

Thermo Scientific

BindIt Software for KingFisher Instruments

User Manual

Software Version 4.0

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Thermo
SCIENTIFIC

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Preface

About This Manual

This guide explains how to install and use Thermo Scientific BindIt™ Software for KingFisher™ instruments version 4.0. You can find information on the software features and operations. The software is used with different types of instruments, and there may be information that may not be relevant to the instrument you are using.

Thermo Scientific™ BindIt™ Software for KingFisher™ instruments is used with:

- Thermo Scientific™ KingFisher™
- Thermo Scientific™ KingFisher™ Duo
- Thermo Scientific™ KingFisher™ Duo Prime
- Thermo Scientific™ KingFisher™ Flex
- Thermo Scientific™ KingFisher™ mL
- Thermo Scientific™ KingFisher™ Presto
- Thermo Scientific™ KingFisher™ 96
- Applied Biosystems™ MagMAX™ Express
- Applied Biosystems™ MagMAX™ Express-96
- Dynal BeadRetriever™

Read the manual in its entirety before using the software.

Related Documentation

In addition to this manual, Thermo Fisher Scientific provides the following instrument-related documents:

- *Thermo Scientific™ KingFisher™ User Manual* (Cat. no. 1507730)
- *Thermo Scientific™ KingFisher™ Duo User Manual* (Cat. no. N12420)
- *Thermo Scientific™ KingFisher™ Duo Prime User Manual* (Cat. no. N16621)
- *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. no. N07669)
- *Thermo Scientific™ KingFisher™ mL User Manual* (Cat. no. 1508260)

Preface

Safety and Special Notices

- *Thermo Scientific™ KingFisher™ Presto User Manual* (Cat. no. N17413)
- *Thermo Scientific™ KingFisher™ 96 User Manual* (Cat. no. 15018890)

The Thermo Scientific™ BindIt™ Software user manual can be found in PDF format in the *Documentation* directory of the installation CD.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual to your local Thermo Fisher Scientific representative.

Software Help

The software Help includes the same information as this manual. To open Help, click the **Help** button or press **F1** on your keyboard.

Use the Help search to find information on specific topics. Write the keyword of the topic you are searching for, click **List Topics**, or press **Enter**.

Safety and Special Notices

Make sure you follow the precautionary statements presented in this guide. The safety and other special notices appear in boxes.

Safety and special notices include the following:



WARNING Risk of injury to the user or users.



CAUTION Risk of damage to the instrument, other equipment or loss of performance or function in a specific application.

Note Marks a hint, important information that is useful for the optimum operation of the system, or an item of interest.

Tip Highlights helpful information that can make a task easier.

Contacting Us

For the latest information on products and services, visit our website at:

www.thermofisher.com/kingfisher.

Contents

	Preface	i
	About This Manual	i
	Related Documentation	i
	Software Help	ii
	Safety and Special Notices	ii
	Contacting Us	ii
Chapter 1	Introduction	1
	BindIt Software	1
	Thermo Scientific KingFisher System	1
Chapter 2	Installing BindIt Software	5
	Installation	6
	Connect the Instrument to the PC	7
	Define a New Instrument	8
	Remove an Instrument from the PC	9
	Uninstall BindIt Software	9
Chapter 3	Main Elements	11
	Application Menu	11
	Navigation Bar	12
	Action Panels	12
Chapter 4	Basic Concepts	13
	Plate Layout	13
	Protocols	15
	Protocols and Runs	15
	Protocol View	15
	Protocol Types	17
	Protocol Compatibility	17
	Run Information	18
Chapter 5	Tip Operation	21
	Tip Combs	21
	Tip Parts	22
	Tip Positions	22

	Tip Speeds	23
	Tip Speed Recommendations	24
Chapter 6	Using BindIt Software	25
	Overview	25
	Start the Software	25
	Open a Protocol	26
	Define a Plate Layout	26
	Plate Layout Types	29
	Duplicate a Well	30
	Duplicate a Plate	30
	Swap Reagent Information	30
	Move Reagent Information	31
	Delete a Plate or Strip	31
	Add Protocol Steps	31
	Modify Protocols	34
	Disable or Enable Steps	35
	Calculate the Duration of a Step or Protocol	36
	Save Protocols	36
	Lock Protocols	37
	Delete Protocols	37
	Execute the Protocol	38
	Add Run Information	38
	Add Sample IDs	39
	Add Lot Information	39
	Lock Run Information	40
	View the Run Report	40
	Export Results	41
Chapter 7	Transfer Protocols	43
	Upload Protocols to the Instrument	43
	Download Protocols from the Instrument	45
	Backups	46
Chapter 8	Protocol Steps and Parameters	49
	Tip	49
	Pick-Up and Leave	49
	Collect Beads	49
	Release Beads	50
	Mix	50
	Beginning of Step	50
	Step Icon	51
	Mixing/Pause Parameters	51
	Mixing/Heating Parameters	52
	End of Step	53
	Dry	53
	Pause	54

Chapter 9	Settings	55
	Options	55
	General	55
	Colors	56
	Laboratory Information	56
	Instruments	56
	Instrument Setup	56
	General	57
	Communications	58
	Automation Interface	59
Chapter 10	Help	61
Chapter 11	Troubleshooting	63
Appendix A	Example Protocol: KingFisher Duo Prime/Duo	65
Appendix B	Example Protocol: KingFisher Flex	73
Appendix C	Effective Use of Software	83

Contents

Introduction

BindIt Software

With BindIt Software you can:

- Create a plate layout.
- Create new and modify existing protocols.
- Transfer protocols to the KingFisher instrument.
- Delete protocols from the KingFisher instrument.
- Execute a protocol directly from the software without sending it to the instrument.
- View protocol status reports.

The protocols and run report files are stored in the file system of the PC. Once a protocol has been created, you can either transfer it to the KingFisher instrument or execute it directly from the software. Protocols run directly from the software are not stored in the instrument memory.

The instrument can store several protocols at the same time. The protocols stored in the instrument can be launched from the KingFisher instrument.

The PC and the instrument must be connected when protocols are transferred to or removed from the instrument. The connection is required also if protocols are executed directly from the software. You can create and edit protocols without an instrument connection.

Thermo Scientific KingFisher System

KingFisher is an automated system for purifying DNA, RNA, proteins and cells from a variety of sample materials, such as blood, cells and tissue samples. It utilizes a patented technology in which magnetic rods move paramagnetic particles through processing steps to purify nucleic acids and proteins, and isolate cells.

The KingFisher purification technology enables high-speed, high-quality processing with no cross-contamination between the samples or reagent carry-over. The KingFisher system provides a complete solution, including instruments, reagents, software and disposable plastics as well as optimized protocols.

The following automated instrument platforms are available for different processing requirements:

- **KingFisher Presto**
 - Purification of 24 or 96 samples per run, maximum capacity thousands of samples per day.
 - A processing volume of 50–5,000 µl with the following three plate formats:
 - 96 KF plate
 - 96 deep well plate
 - 24 deep well plate
- **KingFisher Flex**
 - Purification of 24 or 96 samples in one run, maximum capacity hundreds or thousands of samples per day.
 - A processing volume of 20–5,000 µl with the following four plate formats:
 - 96 KF plate
 - 96 PCR plate, skirted (for example, ABgene SuperPlate #AB-2800)
 - 96 deep well plate
 - 24 deep well plate
- **KingFisher Duo Prime and KingFisher Duo**
 - Purification of 6 or 12 samples in one run. Possibility to run two purification methods sequentially without interruption, raising the throughput to 24 samples. Maximum capacity hundreds of samples per day.
 - A processing volume of 30–5,000 µl with the following formats:
 - 96 deep well Plate
 - 24 deep well Plate
 - KingFisher™ Duo elution strip
- **KingFisher and MagMAX Express**
 - Purification of up to 24 samples in one run, maximum capacity hundreds of samples per day.
 - A processing volume of 20–200 µl in a 96 standard plate.
- **KingFisher mL and BeadRetriever**
 - Purification of 15 samples in one run, maximum capacity hundreds of samples per day.
 - A processing volume of 50–1,000 µl in tubestrips.
- **KingFisher 96 and MagMAX Express 96**
 - Purification of 96 samples in one run, maximum capacity thousands of samples per day.
 - A processing volume of 20–1,000 µl with three different plate formats:
 - 96 KF plate

- 96 PCR plate, skirted (for example, ABgene SuperPlate #AB-2800)
- 96 deep well plate

For more information, see the instrument-related user manuals.

1 Introduction

Thermo Scientific KingFisher System

Installing BindIt Software

This chapter contains information on installation of BindIt Software.

To install BindIt Software you need:

- Administrator rights to the PC.
- The BindIt Software installation CD.
- To make sure that your PC meets the minimum requirements.

Note Failure to follow these instructions may lead to an unsuccessful installation of BindIt Software.

Table 1. Minimum PC requirements

Minimum PC requirements	
Interface	USB port or RS-232 serial communication port, depending on the instrument model
Supported operating systems	Microsoft™ Windows™ 7 with Service Pack 1 (32- or 64-bit), Microsoft™ Windows™ 8.1 (32- or 64-bit) and Microsoft™ Windows™ 10 (32- or 64-bit)
Disk space	0.5 GB free disk space
Processor	Intel Pentium (or equivalent), 1 GHz or faster
Memory	1 GB RAM
Serial or USB ports available	1
Pointing device	Mouse or equivalent necessary
CD-ROM drive	1
Monitor / Color settings	XVGA monitor with 1024 x 768 resolution

Tip If you do not have the correct Service Packs installed, download them from the Microsoft™ website at: <http://www.microsoft.com>

Installation

This section describes the software installation procedure.

Note BindIt Software cannot be installed on a network drive.

Note You must be logged on to your computer with administrator privileges to install BindIt Software.

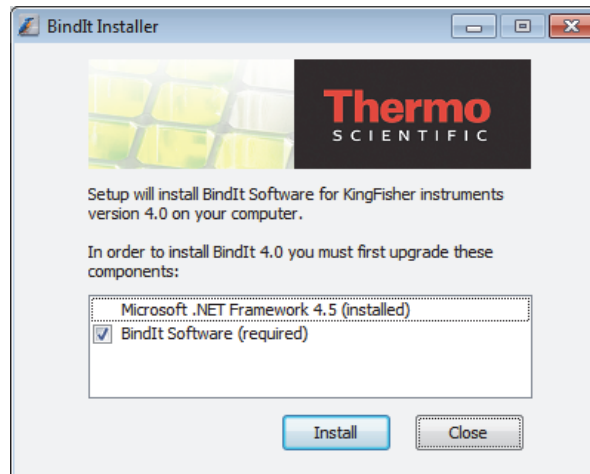
Note You can stop the installation procedure at any stage by clicking **Cancel**. The setup rolls back your system to the initial state.

To install BindIt Software:

1. Insert the installation CD into the CD-ROM drive.

The BindIt Installer dialog opens.

Figure 1. BindIt Installer



If the dialog does not open, open the Setup file from the CD.

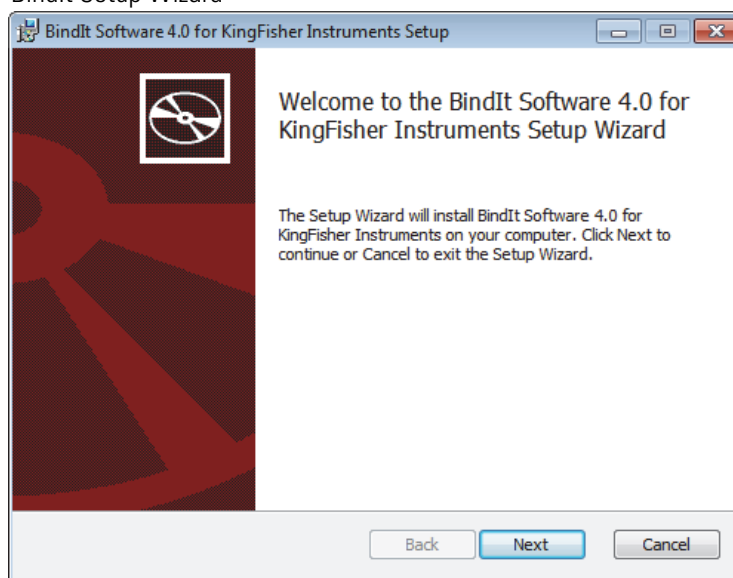
2. In the components list dialog, verify the selected the components, and click **Install**.

Note If you are asked to restart your computer during the installation, confirm the restart. On a Windows 7 (32-bit) PC, the installation may not continue automatically after the restart. If this occurs, continue the installation by double-clicking the *Setup.exe* file on the installation CD.

After the required prerequisites are installed, the BindIt Software setup wizard opens.

3. The setup wizard guides you through the installation procedure. Click **Next**.

Figure 2. BindIt Setup Wizard



4. Read the end user license agreement and tick the box to accept the terms.
If you do not accept the license agreement, the installation does not continue.
5. Click **Next**.
6. Select the destination folder for the BindIt Software installation files.
The Setup Wizard suggests a location for the files. Using the default file location is recommended.
7. Click **Next**.
8. Click **Install**.
9. Click **Finish**.
10. The Setup Wizard shows an “Application installed successfully.” message. Click **OK**.
BindIt Software has now been installed.

If you want to connect the software to an instrument, refer to [“Connect the Instrument to the PC”](#) on [page 7](#).

If you do not set up a connection to any instrument at this point, the simulator is set as the default instrument.

Connect the Instrument to the PC

After installing the software, connect the instrument to the PC with a USB cable or an RS-232 C serial cable (COM).

Note KingFisher and KingFisher mL / BeadRetriever with firmware version lower than 2.0 and KingFisher 96 / MagMAX Express 96 do not work with a USB port.

1. Ensure that the instrument is switched off.
2. Connect the instrument to the PC by using a USB or COM cable.

3. Switch on the instrument. Wait for the instrument to initialize.
4. Launch BindIt Software.

If you used a USB cable, BindIt Software finds the instrument automatically.

If you used a COM cable, define the new instrument manually in the **Settings** of BindIt Software

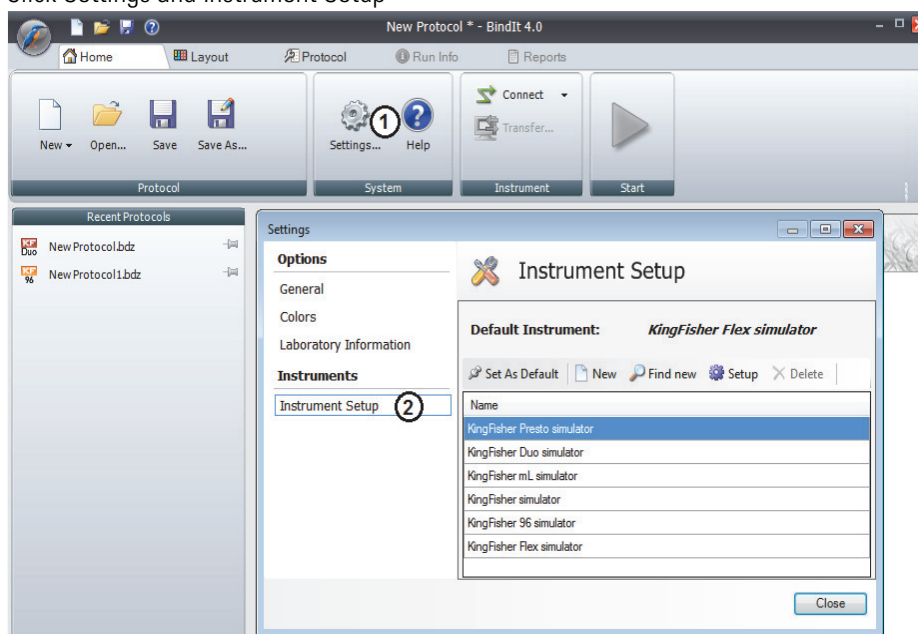
For more information on connecting instruments, refer to “[Instrument Setup](#)” on [page 56](#).

Define a New Instrument

To add a new instrument to BindIt Software:

1. Click **Settings**.
2. Click **Instrument Setup**.

Figure 3. Click Settings and Instrument Setup



3. Click **New**.
4. Name the instrument, select the instrument type from the list, and type the serial number.
5. Go to the **Communications** tab.
6. In the Communication field, select the type of cable you are using and fields (see “[Communications](#)” on [page 58](#)).
7. Click **OK**.
8. In the *Instrument Setup* dialog, select the new instrument from the *Instruments* list, and click **Set As Default**.
9. Click **Close**.

Note You can add several new instruments before clicking **Close**.

In the **Home** view, you can connect to the new instrument by clicking **Connect**, and selecting the instrument from the list.

For more information on connecting instruments, refer to “[Instrument Setup](#)” on [page 56](#).

Remove an Instrument from the PC

You cannot delete the default instrument from the software. If you want to delete the instrument that has been selected as a default instrument, first set another instrument as a default instrument.

1. Go to **Instrument Setup** under **Settings**.
2. Select the instrument that you wish to remove from the list.
3. Click **Delete**.
4. Click **Yes** to confirm the deletion.

Uninstall BindIt Software

To uninstall BindIt Software:

1. Open **Control Panel** from the Windows **Start** menu.
2. Select **Programs and Features**.
3. Select **BindIt Software 4.0 for KingFisher** from the program list.
4. Click **Uninstall**.
5. Click **Yes** to confirm the deletion.

BindIt Software is uninstalled.

2 Installing BindIt Software

Uninstall BindIt Software

Main Elements

This chapter describes the main elements and views of the BindIt Software user interface (UI).

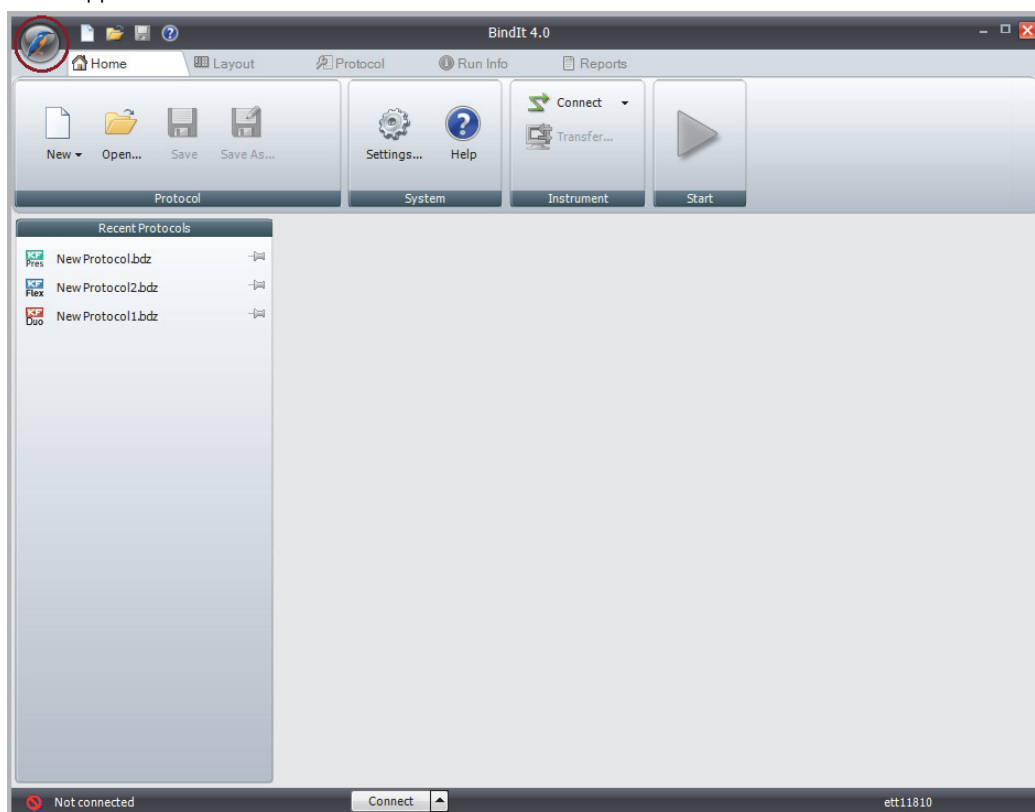
When you log in to BindIt Software, the main view (**Home**) opens.

Each view displays the **Application menu**, **Action panels** and **Navigation bar**.

Application Menu

The application menu is for general tasks. This is where you create new sessions, open saved sessions and access instrument and software settings. Click the menu icon to open the application menu.

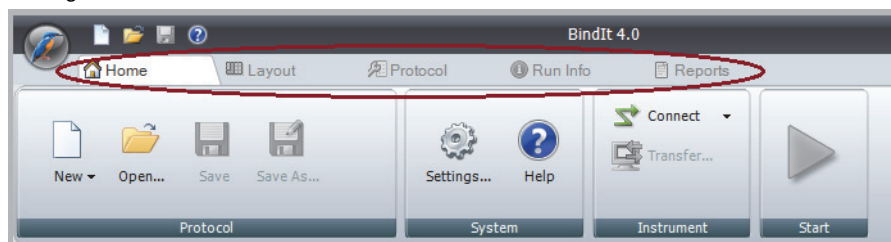
Figure 1. Application menu



Navigation Bar

This is where you navigate between different views. The user interface is divided into five main views: **Home**, **Layout**, **Protocol**, **Run Info**, and **Reports**. When you select a tab from the navigation bar, the relevant view with its action panels opens.

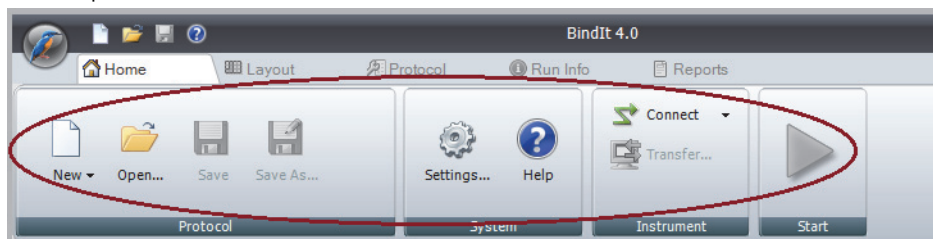
Figure 2. Navigation bar



Action Panels

The action panels show the actions you can select. The actions are linked to the main views you can select in the navigation bar.

Figure 3. Action panels



Basic Concepts

This chapter describes the main concepts of BindIt Software.

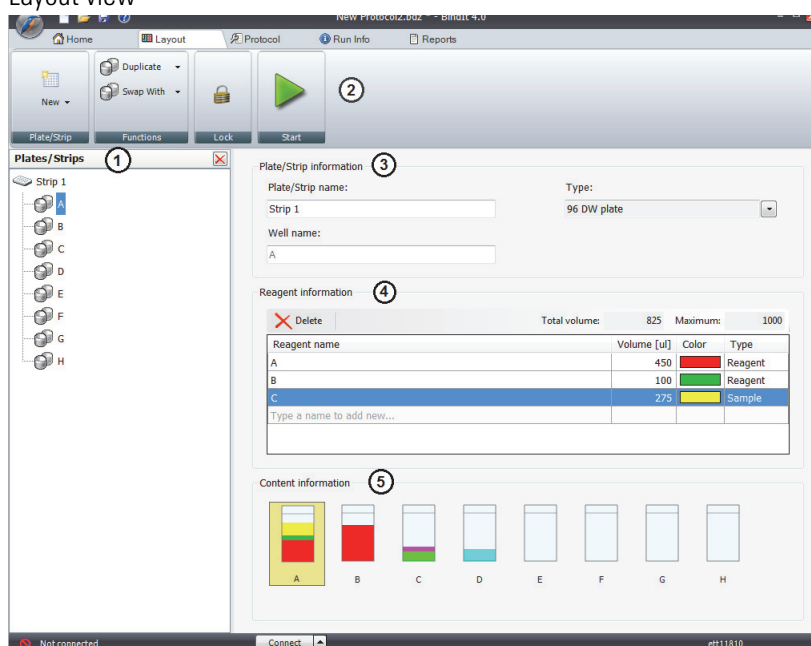
Plate Layout

The Layout view is where you define the plate content. You select the plate template, define the sample types that you have, and tell the software which wells to measure.

Plate layouts and their creation depends on the target instrument.

Click the **Layout** tab to open the **Layout** view:

Figure 4. Layout view



Item	Description
1	The Plates/Strips tree displays the plates/strips in the plate layout.
2	Action panels (see below)
3	The Plate/Strip information field is for the name of each plate/strip.
4	The Reagent information field is for reagent details for each plate/well.
5	The Content information field displays the proportions of the reagents. The summary is also visible in the Home view.

Figure 5. The Layout ribbon

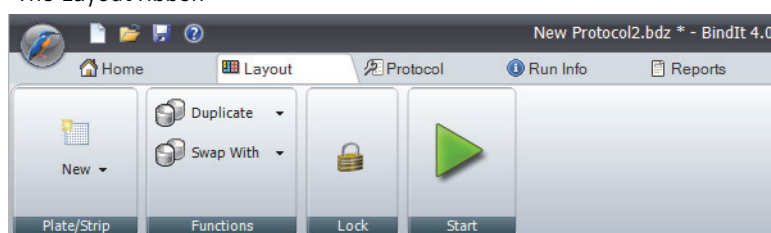


Table 1. Layout view action panels

Action	Description
New Plate/Strip	Shows a list of available plates or strips from which you can select one for a new plate. The new plate appears in the Plates/Strips tree on the left side of the window.
Duplicate	Adds a copy of the selected plate to the tree view or overwrites the information of an existing well.
Swap with	Changes the reagent information of two strips with each other.*
Move from	Moves the reagent information of the selected empty strip.*

Table 1. Layout view action panels

Action	Description
Lock	Locks the protocol so that it can no longer be modified. After you have locked the protocol, you cannot unlock it.
Start	Executes the protocol.

* This function is available only for KingFisher Duo Prime/KingFisher Duo, KingFisher mL/BeadRetriever, and KingFisher/MagMAX Express.

Protocols

Protocols and Runs

In BindIt Software, *protocol* refers to a collection of information regarding the layout with reagent data and procedure steps performed in a session as well as the instrument used. Such a protocol can be saved for use whenever it is required.

When you start a protocol, it creates a separate *run*. *Run-specific* information such as sample data and consumable lot numbers can be entered in the **Run Info** view before, during or after protocol execution. A green triangle designates a protocol that has been executed. All protocols and run reports are stored in the file system (see [Figure 10](#)). Protocol file extension is .bdz and run report file extension is .KRun.

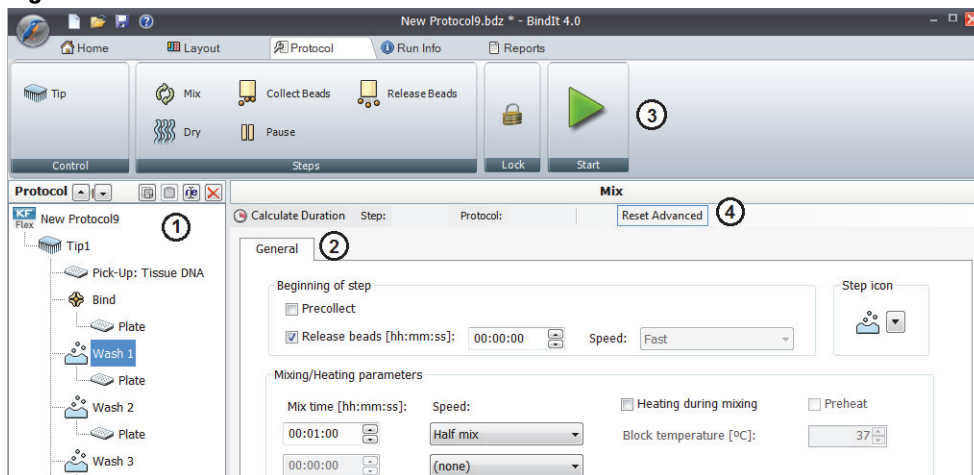
Both protocols and run information can be locked to prevent accidental modification. A locked protocol or run cannot be opened, but a new copy of it can be created.

Note Before locking a protocol, we recommend validating it with an instrument and actual samples and reagents.

Protocol View

The following figure shows the different parts and functions of the **Protocol** view. For instructions on how to use these functions, see [“Add Protocol Steps”](#) on [page 31](#).

Figure 6. Protocol view



Item	Description
1	Tree view
2	Main pane
3	Action panels
4	Show Advanced

The **Tree view** includes the following protocol steps:

Table 2. Tree view

Icon	Description
	Move step up moves steps up in the tree view.
	Move step down moves steps down in the tree view.
	Copy step copies a step in the tree view
	Paste step pastes a step in the tree view.
	Rename step allows you to rename a step.
	Delete step removes a step from the protocol.

Main pane shows the step parameters.

Action panels include the following functions:

Table 3. Protocol view action panels

Action	Description
Tip	Adds a new tip comb to the Protocol Steps tree view.
Mix	Adds a step to the protocol for mixing, heating, binding, washing and eluting the reagents according to given parameters.

Table 3. Protocol view action panels

Action	Description
Collect Beads	Adds a step to the protocol for collecting beads from the samples or reagents in the well/tube of the plate.
Release Beads	Adds a step to the protocol for releasing beads into a well/tube of the plate.
Dry	Adds a step to the protocol for drying the tip.
Pause	Pauses the protocol for manual handling.
Start	Executes the protocol.

Show Advanced shows all parameter fields in the *Mix* step. **Reset Advanced** hides some of the fields and returns the parameter values back to default.

Protocol Types

There are two types of protocols, user and factory protocols. User protocols are the protocols you create for BindIt Software whereas factory protocols are ready-made protocols by Thermo Fisher Scientific.

Factory protocols can be divided further into two categories: demo and application protocols. Demo protocols are supplied with BindIt Software as examples and they are located in the file system: `Public Documents\BindIt Factory protocols`. Application protocols can be downloaded from www.thermofisher.com/kingfisher.

Protocol Compatibility

If you have older protocols from previous versions of BindIt Software or KingFisher Software that you would like to use with BindIt Software 4.0, see the actions you need to take:.

Table 4.

File extension	Older software versions	Action(s)
.bdz	BindIt Software 3.3.1, 3.3 and 3.2	No conversion needed for BindIt Software 4.0. Use the Export functionality of BindIt Software 3.3.1, 3.3 or 3.2 to export the protocols to the file system of your PC.

Table 4.

File extension	Older software versions	Action(s)
.msz	BindIt Software 3.1 and 3.0	Convert to .bdz files using BindIt Software 3.3.1, 3.3 or 3.2: Import the .msz file to BindIt Software 3.3.1, 3.3 or 3.2 which converts the protocol to a .bdz file. Then use the Export functionality to move the newly created .bdz file to the file system of your PC.
.kf2	KingFisher Software 2.6.2	Convert to .bdz files using BindIt Software 4.0: Open the .kf2 protocol with BindIt Software 4.0, and save it using the Save As function. This sets all the speed parameters to medium speed (see Note 1). See Table 7 for the mixing speeds. **Note 1 To see the original speed parameters of a .kf2 protocol, open the protocol in an older version of BindIt Software or in KingFisher Software 2.6.2.w
<p>Note You need BindIt Software 3.3.1, 3.3 or 3.2 to convert .kf2 and .msz files to .bdz files. BindIt 4.0 can be installed simultaneously with BindIt 3.x or KingFisher Software 2.6.2.</p>		
<p>Note BindIt 4.0 can be installed and used simultaneously with BindIt 3.x or KingFisher Software 2.6.2. However, you cannot connect both applications to the same instrument at the same time.</p>		

Run Information

The **Run Info** view allows you to add run-specific information. This information can include sample IDs and replicates, as well as data on the used consumables and their lot numbers.

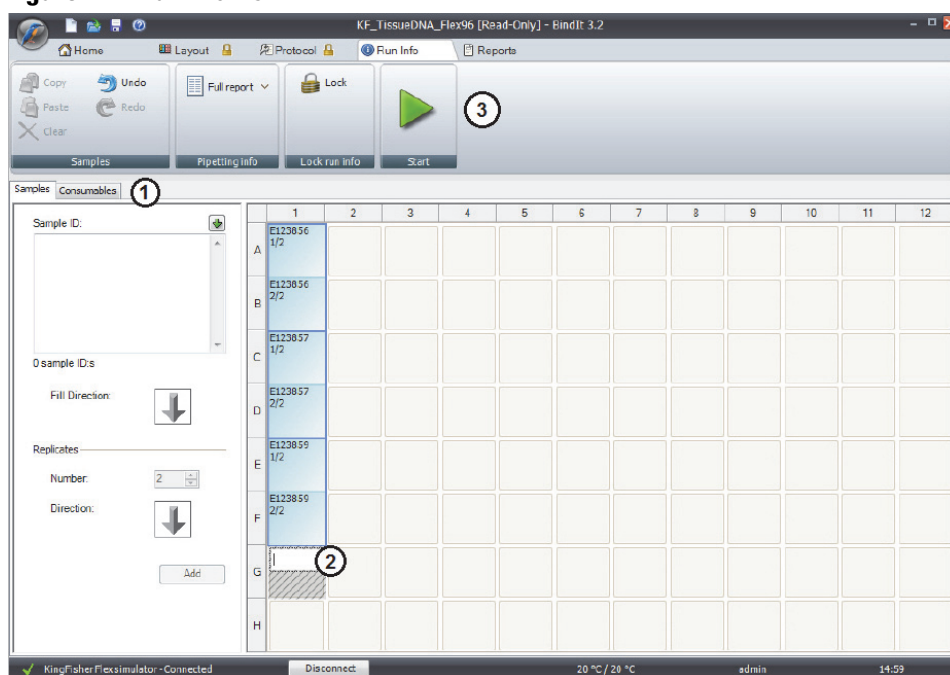
Run information can be entered before, during or after protocol execution, but it is typically entered when the protocol execution has been started.

Run-specific information can also be entered and saved for execution at a later time. The entered data is stored as part of the run when it is executed. Executed runs are indicated by green triangles.

This feature is not available for KingFisher Presto.

The following figure shows the different parts and functions of the **Run Info** view.

Figure 7. Run Info view



Item	Description
1	Tabs
2	Sample ID
3	Action panels

Action panels include the following functions:

Table 5. Run Info view action panels

Action	Description
Copy	Copy the selected sample data.
Paste	Select the copied sample data to the selected location.
Clear	Clear the selected sample data.
Undo/Redo	Undo or redo changes to the sample data.
Full report	Create a full run information report or select the sections to be included in the report.
Lock	Lock run information so that it can no longer be modified. After you have locked run information, you cannot unlock it any more.
Start	Executes the protocol.

Tip Operation

This chapter describes the different types of tip combs you can use with the KingFisher instruments. It also includes the parts and settings of the tip combs.

Tip Combs

KingFisher/MagMAX Express and KingFisher mL/BeadRetriever have one specially designed tip comb. You must install the tip comb into the instrument manually.

If you are using or KingFisher Presto, KingFisher Duo Prime/KingFisher Duo, KingFisher Flex or KingFisher 96/MagMAX Express 96, the tip comb type is selected when creating a new protocol. You can change the tip comb type from the list in the *Tip Selection* dialog in the **Protocol** view.

The following table shows the available tip combs:

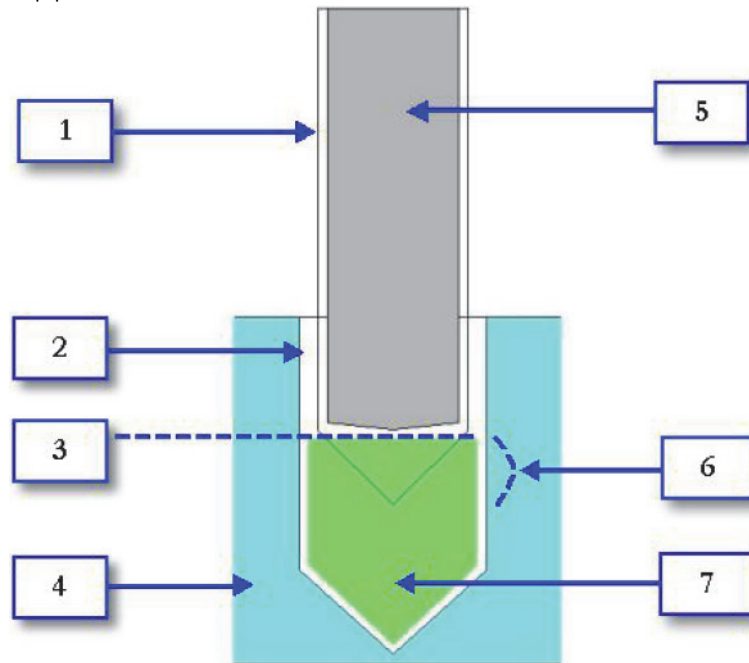
Table 6. Tip combs

Instrument	Tip comb
KingFisher Presto	24 deep well tip comb
	96 deep well tip comb
KingFisher Duo Prime and KingFisher Duo	KingFisher Duo 12-tip comb
	KingFisher Duo 6-tip comb
KingFisher Flex	24 deep well tip comb
	96 tip comb
	96 deep well tip comb
	PCR tip comb
KingFisher 96 and MagMAX Express 96	96 deep well tip comb
	PCR tip comb
	96 tip comb

Tip Parts

The following figure shows the different parts of the tip inside a well.

Figure 8. Tip parts

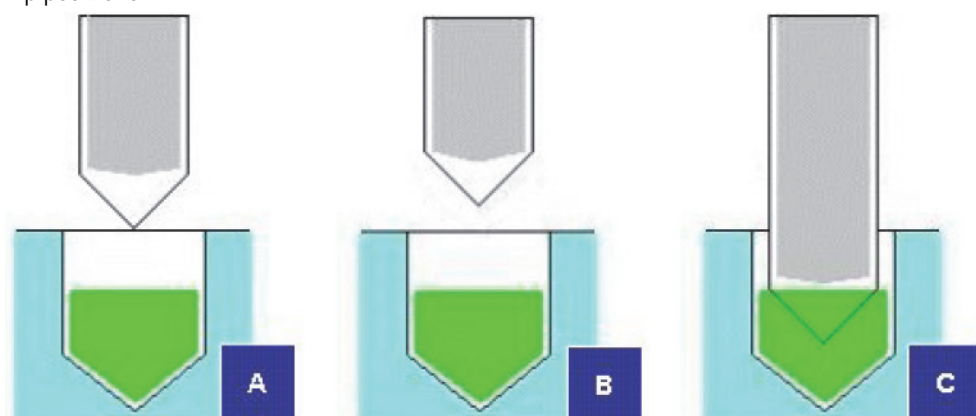


Item number	Description	Item number	Description
1	Plastic tip	5	Magnetic rod
2	Well	6	Tip edge
3	Liquid surface level	7	Liquid
4	Plate cross-section		

Tip Positions

The tip position varies during the protocol depending on the protocol steps and settings.

The different tip positions are described in the following figure:

Figure 9. Tip positions

Item	Description
A	The tip is above a well or tube (at the well or tube surface but not touching the liquid).
B	The tip is outside a well or tube.
C	The tip edge is in the liquid.

Tip Speeds

There are two types of speeds for the plastic tip movement in the well/tube: full-length speed (*Slow*, *Medium* and *Fast*) and bottom mix speed (*Bottom mix* and *Half mix*). In the full-length speeds, the tip moves throughout the length of the well, whereas in the bottom mix speeds, the tip moves only at the bottom of the well.

As a general rule, the *Medium* tip speed is half of the *Fast* speed, and *Slow* is one tenth of the *Medium* tip speed.

In the bottom mix speed, the tip has a rapid and short movement close to bottom of the well. The bottom mix is effective for small volume mixing and for dispersing the possible magnetic bead clumps.

In the half mix speed, the tip moves approximately half the height of the reagent column. The movement is fast and suitable, for example, for efficient mixing of large volumes or in a combined loop with the fast or bottom mix speeds.

The length of the movement and the tip speed vary according to the volume of the liquid in the well/tube. This is to prevent splashing.

If you have upgraded from KingFisher Software 2.6.2 to BindIt Software, the following table presents the relation of mixing speeds between the software versions.

Table 7. Relation of mixing speeds between software versions.

KingFisher Software 2.6.2	BindIt Software
Very slow	Slow
Slow	

Table 7. Relation of mixing speeds between software versions.

KingFisher Software 2.6.2	BindIt Software
Medium	Medium
Fast	
Very fast	Fast
Super fast	
Half mix	Half mix
Grind mix	Bottom mix
Bottom very slow	Medium
Bottom slow	
Bottom medium	Bottom mix
Bottom fast	

Tip Speed Recommendations

Consider the following general rules and recommendations when selecting tip speeds:

- Use the *Medium* speed as a starting point if you are unsure of speed selection. If the beads do not seem to mix, try a higher speed or different speed combinations.
- You can use all available speeds and speed combinations. The most effective speeds are *Fast*, *Half mix*, and *Bottom mix*. You can also loop these speeds for very effective results.
- If the beads stay at the bottom of the well/tube, the speed is too low. If the sample and the particles form a clump, it can be dissolved by selecting the *Bottom mix* speed. Continue with *Half mix* or *Fast* to mix the beads in the liquid.
- Use the **Precollect** parameter in a step if the beads are sedimented at the bottom at the beginning of the step.
- By using the *Slow* or *Medium* speeds for heating during mixing, the liquid heats up faster and does not cool down during the step.
- If you wish to wash the beads with water, for example, after washing them with ethanol, use the *Slow* speed and do not release the beads.
- Use *Fast*, *Medium* or *Slow* for *Elution*. Test different speeds to find the most suitable one for the protocol.
- Make sure that the selected liquid, volume and speed combination does not cause splashing, which can lead to cross-contamination.

Using BindIt Software

This chapter shows how to define the layout and the protocol steps, and add optional run-specific information, as well as how to manage protocols.

Overview

When using BindIt Software, you generally either open an existing protocol or create a new protocol. You can also modify existing protocols.

The general outline for using the software:

1. [Start the Software](#).
2. [Open a Protocol](#) that already exists or create a new protocol by following steps 3-6.
3. [Define a Plate Layout](#).
4. [Add Protocol Steps](#).
5. Start to [Execute the Protocol](#).
6. [Add Run Information](#), if necessary
7. [View the Run Report](#) for the results.
8. [Export Results](#).

Start the Software

To launch the software, double-click the **BindIt 4.0 for KingFisher** icon on your desktop.

The software tries to connect to the default instrument if the **Connect to default instrument at startup** is selected from **General** under **Settings**. When BindIt Software is launched, it searches automatically for new USB-connected KingFisher devices. Otherwise, click **Connect** and select the target instrument from the list.

For more information on connecting instruments, see [“Define a New Instrument”](#) on [page 8](#).

Note If the software installation contains a newer version of the instrument firmware, you are prompted to update the firmware when connecting to the instrument.

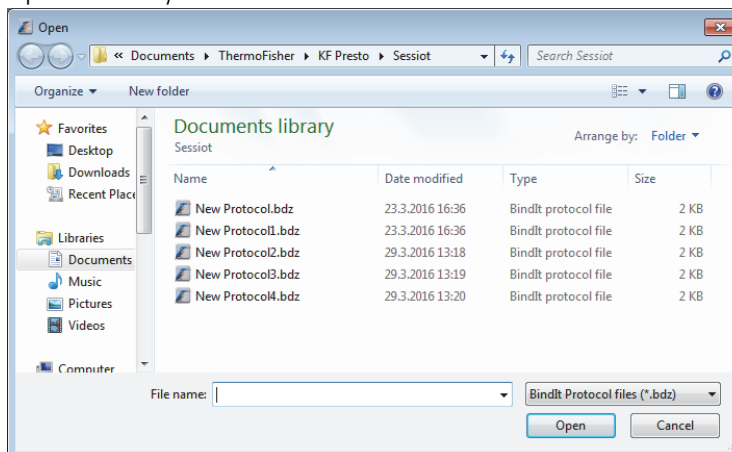
Open a Protocol

To open an existing protocol for use or editing:

1. In the **Home** view, click **Open**.

The file system opens. By default only the protocol files (*.bdz) are shown.

Figure 10. Open the file system.



2. Select the desired protocol or run.

3. Click **Open**.

The opened protocol or run can be used as is, if it contains the necessary information or it can be used as a basis for creating a new protocol.

Note To edit protocols created with KingFisher Software 2.6.2, open the protocol and save it by using the **Save As** function. This saves the protocol as a BindIt protocol, after which you can edit the protocol freely. In this new BindIt protocol, all speed parameters are set to medium speed.

Define a Plate Layout

The following procedures are presented as examples of creating a plate layout. Use them as a starting point for creating your own protocols. We recommend that practice by going through an example before creating or editing your own plate layouts.

There are two different ways to create a plate layout depending on the instrument you are using.

If you are using one of the following, follow the **first** set of instructions:

- KingFisher Duo Prime/KingFisher Duo
- KingFisher/MagMAX Express
- KingFisher mL/BeadRetriever

Select the **second** set of instructions, if you are using one of the following:

- KingFisher Presto
- KingFisher Flex

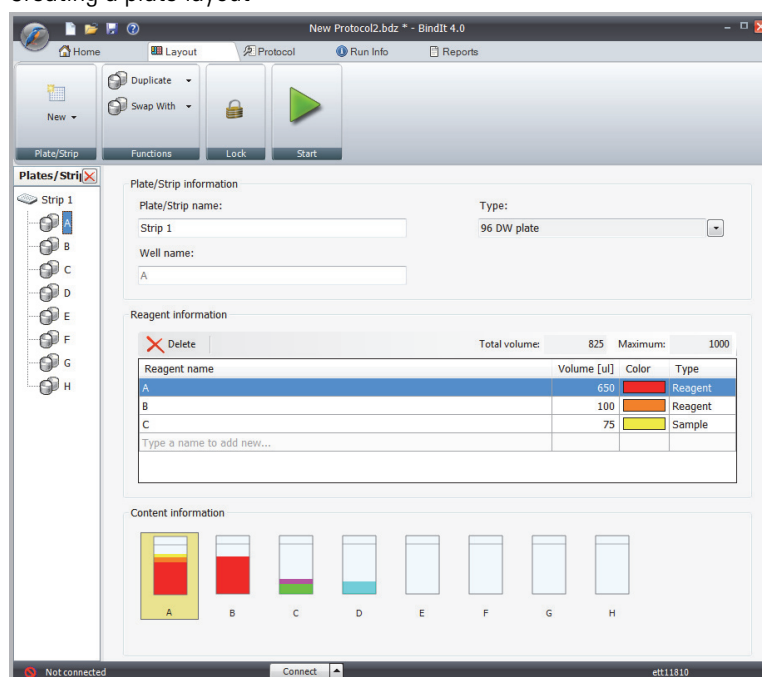
- KingFisher 96/MagMAX Express 96

You can also refer to the layout creation examples in [Appendix A, “Example Protocol: KingFisher Duo Prime/Duo.”](#) and [Appendix B, “Example Protocol: KingFisher Flex.”](#)

❖ **Define a plate layout for KingFisher Duo/KingFisher Duo Prime, KingFisher/MagMAX Express and KingFisher mL/BeadRetriever**

1. In the **Home** view, click **New** and select the instrument type from the drop-down menu.
2. Click **New** in the **Layout** view and select the plate type from the **Plate/Strip** list.

Figure 11. Creating a plate layout



3. In the *Plate/Strip information* field, specify the plate/strip name and press **Enter**.

Note The name is required for protocol creation.

4. In the **Strips** tree view, click the first well (A).
5. In the *Plate/Strip Information* field, name the tube/well.
6. In the *Reagent information* field, specify the reagent details:

Tip Write each reagent in a well on a separate row.

- a. Type the reagent name.
- b. Insert the volume of the reagent.

Check the accepted volumes from [Table 8](#).

In addition, the *Maximum* field displays the allowed maximum volume range for the selected plate type and the *Total volume* field displays the total volume of the added reagents.

- c. Select the reagent color to be shown in the proportional reagent view.
- d. Select the reagent type (*Reagent* or *Sample*).

Rows marked as *Reagents* are shown in the list of consumables in the **Run Info** view, whereas *Samples* are not.

To delete a reagent row, click **Delete**.

Tip In the *Protocol Summary* field of the **Home** view, you can view the reagent information of a strip or a plate. A tooltip appears when you move the cursor over the desired strip/plate.

7. Type the name and specify the reagent details also for the other wells or tubes in the plate or strip.

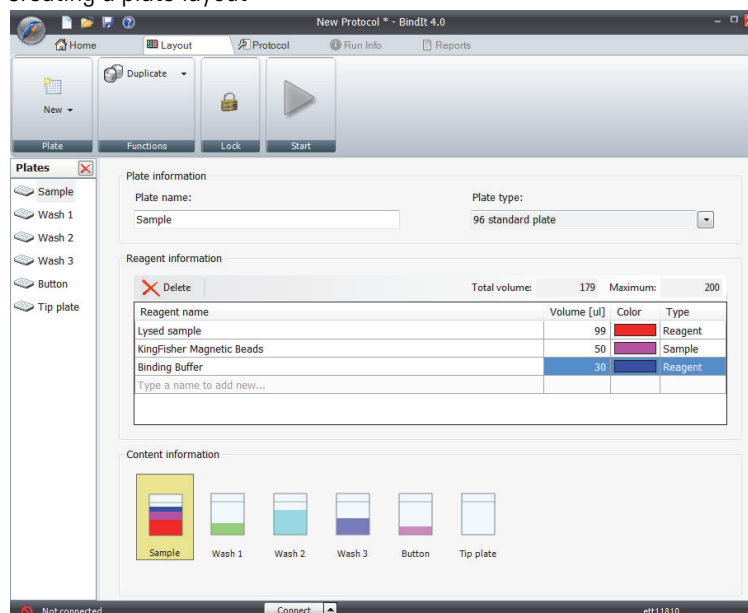
You can see the proportions of each reagent in the *Content information* field of the **Layout** view, or in the *Protocol summary* field in the **Home** view.

❖ **Define a Plate Layout for KingFisher Presto, KingFisher Flex and KingFisher 96 / MagMAX Express 96**

1. In the **Home** view, click **New** and select the instrument type from the drop-down menu.

2. Click **New** in the **Layout** view and select the plate type from the list.

Figure 12. Creating a plate layout



3. In the *Plate name* field, specify the plate name and press **Enter**.

Note The name is required for protocol creation.

4. In the *Reagent information* field, specify the reagent details:

Tip Add each reagent to a separate row.

- a. Type the reagent name.
- b. Insert the volume of the reagent.
Check the accepted volumes from [Table 8](#).
- c. Select the reagent color to be shown in the *Content information* field.
- d. Select the reagent type (*Reagent* or *Sample*).

Rows marked as *Reagents* are shown in the list of consumables in the Run Information view, whereas *Samples* are not.

To delete a reagent row, click **Delete**.

5. Type the name and specify the reagent details also for the other wells or tubes in the plate or strip.

You can see the proportions of each reagent in the *Content information* field of the **Layout** view, or in the *Protocol summary* field in the **Home** view.

Tip In the *Protocol Summary* field of the **Home** view, you can view the reagent information of a strip or a plate. A tooltip appears when you move the cursor over the desired strip/plate.

Plate Layout Types

The KingFisher instruments handle different types of plates or tube strips with a different number of wells/tubes with different volumes. A single protocol can use several different plate types. Ultimately, every step can be assigned to a different plate layout if necessary.

Table 8 shows the available plate layouts for the instruments. The software does not accept volumes which exceed the maximum values.


Table 8. Plate layouts for KingFisher instruments

Instrument	Layout	Volume range
KingFisher Presto	24 DW plate	200 µl – 5 ml
	96 DW plate	50–1,000 µl
	96 plate with 96 DW tip comb	50–150 µl
KingFisher Duo Prime/KingFisher Duo	KingFisher Duo elution strip	30–130 µl
	96 DW plate	50–1,000 µl
	24 DW plate	200 µl – 5 ml
KingFisher Flex	96 standard plate with 96 tip comb	20–200 µl
	96 standard plate with 96 DW tip comb	50–150 µl
	96 DW plate	50–1,000 µl
	96 PCR plate, skirted	20–100 µl
	24 DW plate	200 µl – 5 ml
KingFisher/MagMAX Express	Strip plate 100 µl (default)	20–100 µl
	Strip plate 200 µl	20–200 µl
KingFisher mL/BeadRetriever	Tubestrip 1,000 µl (default)	50–1,000 µl
	Microtube 1.5 mL	500–600 µl
	Microtube 1.9 mL	500–800 µl

Table 8. Plate layouts for KingFisher instruments

Instrument	Layout	Volume range
KingFisher 96/MagMAX Express 96	96 standard plate (default) with 96 tip comb	20–200 µl
	96 standard plate (default) with 96 DW tip comb	50–150 µl
	96 DW plate	50–1,000 µl
	96 PCR plate, skirted	20–100 µl



CAUTION Do not exceed the maximum volumes. The total volume of all reagents in a well/tube must always remain within the volume range given in the table. If the maximum volume is exceeded, the notification  appears. If the volume exceeds the maximum by more than 30%, the software does not allow you to execute the protocol. However, the software accepts volumes smaller than the minimum volume. If the total volume is smaller than the minimum, the magnetic rod does not reach the liquid.

Duplicate a Well

In KingFisher Duo Prime/KingFisher Duo, KingFisher mL/BeadRetriever and KingFisher/MagMAX Express, **Duplicate** overwrites the information of the existing well.

1. In the **Layout** view, select the well in the tree view to which you wish to copy the reagent data.
2. Click **Duplicate**, and select from the drop-down menu the well, the information of which you want to copy.
3. Change the name of the well and edit other fields if necessary.

Duplicate a Plate

In KingFisher Presto, KingFisher Flex and KingFisher 96/MagMAX Express 96, **Duplicate** adds a new plate to the protocol.

1. In the **Layout** view, click **Duplicate** and select the plate that you want to copy from the drop-down menu.
A new plate is added to the bottom of the tree view.
2. Change the name of the plate and edit other fields if necessary.

Swap Reagent Information

The **Swap With** function changes the reagent information of two wells/tubes with each other.

This function is available only for KingFisher Duo Prime/KingFisher Duo, KingFisher mL/BeadRetriever and KingFisher/MagMAX Express.

1. In the **Layout** view, select a tube/well in the **Plates/Strips** tree view.

2. Click **Swap With**, and select the tube/well, the information of which you want to change.

Move Reagent Information

The **Move From** function moves the reagent information from a well/tube to an empty well/tube.

This function is available only for KingFisher Duo Prime/KingFisher Duo, KingFisher mL/BeadRetriever and KingFisher/MagMAX Express.

1. In the **Layout** view, select an empty tube/well in the **Plates/Strips** tree view.
2. Click **Move From**, and select the tube/well, the information of which you want to move to another well.

Delete a Plate or Strip

To remove plates or strips from the layout:

1. In the **Layout** view, select the plate or strip in the *Plates/Strips* tree view.
2. Click **Delete**.
3. Click **Yes** to confirm the deletion.

Add Protocol Steps

In general, you can add steps to a protocol by clicking a step in the **Steps** action panel of the **Protocol** tab, or by right-clicking in the *Protocol Steps* tree and selecting the step from the context menu.

Note The maximum number of steps to add to a KingFisher Presto protocol is approximately 60. The number varies depending on the steps and selected options. If the protocol is too large, KingFisher Presto gives an error message that you cannot load the protocol.

You can move a protocol step by selecting it and using the up and down arrow buttons above the *Protocol Steps* tree. This command bar provides tools also for copying, pasting, renaming and deleting steps.

The following procedures present the creation of new protocols, but the instructions apply also to modifying existing protocols.

There are two different ways to add protocol steps depending on the instrument you are using.

If you are using one of the following, follow the **first** set of instructions:

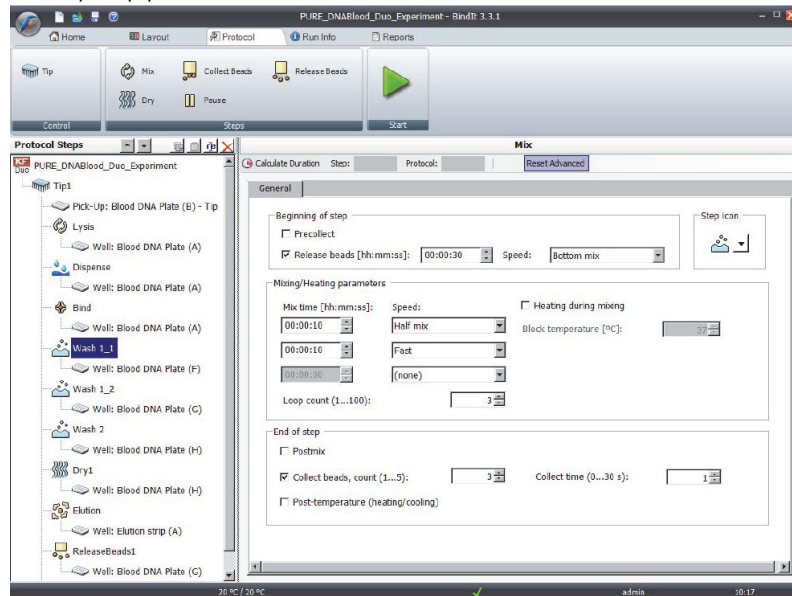
- KingFisher Duo Prime/KingFisher Duo
- KingFisher/MagMAX Express
- KingFisher mL/BeadRetriever

Select the **second** set of instructions, if you are using one of the following:

- KingFisher Presto

- KingFisher Flex
 - KingFisher 96/MagMAX Express 96
- ❖ **Add protocol steps to KingFisher Duo Prime/KingFisher Duo, KingFisher mL /BeadRetriever and KingFisher/MagMAX Express**
1. In the **Protocol** view, click the name of the protocol in the *Protocol Steps* tree and name the kit used in the protocol in the *Kit name* field.
 2. Type a free-form description of the protocol in the *Protocol description* field.
 3. Click **Tip** and select the tip comb from the *Tip* field.
 4. (KingFisher Duo Prime/Duo only) Select the row from which the tip comb is picked up by clicking **Pick-Up** in the *Protocol Steps* tree, and by selecting the row from the *Pick-up* from list.
 5. Add a step to the protocol by selecting it from the **Steps** action panel.
 6. Click the step to enter or modify its parameters.

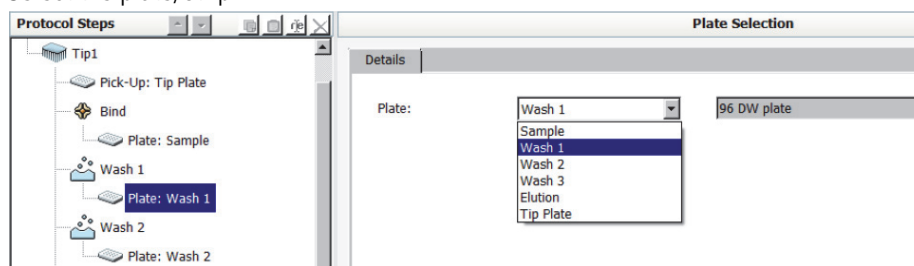
Figure 13. Modify step parameters



Note In the *Mix* step, you can see all available parameter options by clicking **Show Advanced**. See Chapter 8, “Protocol Steps and Parameters.” for details of the step parameters.

7. Click the tube or well under the step in the tree.
8. In the *Plate Selection* dialog, select the plate or strip from the *Plate/Strip* menu.

Figure 14. Select the plate/strip



9. Select the well from the *Well* list.
10. Repeat Steps 5.–9. to add steps to the protocol.
11. (KingFisher Duo Prime/Duo) After you have added all the required steps, select the row to which the tip comb is left at the end of the protocol by clicking **Leave** and selecting the plate from the Plate menu. It is recommended to leave the tip comb on the same row from which it was picked up.

Add more tip combs to the protocol after the last step, if necessary.

❖ **Add Protocol Steps to KingFisher Presto, KingFisher Flex and KingFisher 96 / MagMAX Express 96**

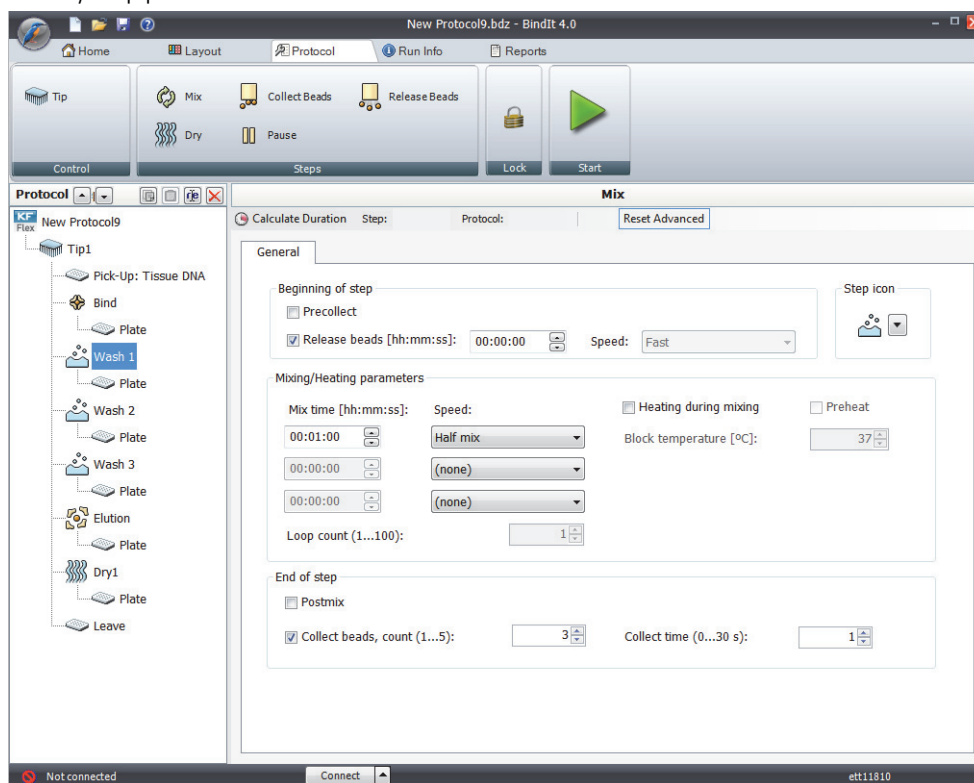
1. In the **Protocol** view, click the name of the protocol in the *Protocol Steps* tree and type a name for the kit in the *Kit name* field.
2. Type a free-form description of the protocol in the *Protocol description* field.
3. Click the **Tip** icon in the tree view.
4. Select the tip comb type from the *Tip* list.



CAUTION Ensure that the tip comb type matches the plate type

5. Select the plate from which the tip comb is picked up by clicking **Pick-Up** in the *Protocol Steps* tree, and by selecting the plate from the *Plate* list.
6. Add a step to the protocol by selecting it from the **Steps** action panel, or by right-clicking the *Protocol Steps* tree and selecting the step.
7. Click the step to enter or modify its parameters.

Figure 15. Modify step parameters



Note In the *Mix* step, you can see all available parameter options by clicking **Show Advanced**. See [Chapter 8, “Protocol Steps and Parameters.”](#) for details of the step parameters.

8. In the tree view, click **Plate**.
9. In the *Plate Selection* dialog, select the plate from the *Plate* drop-down menu.
10. After you have added all the required steps, select the plate to which the tip comb is left at the end of the protocol by clicking **Leave** and selecting the plate from the *Plate* menu.

Add more tip combs to the protocol after the last step if necessary.

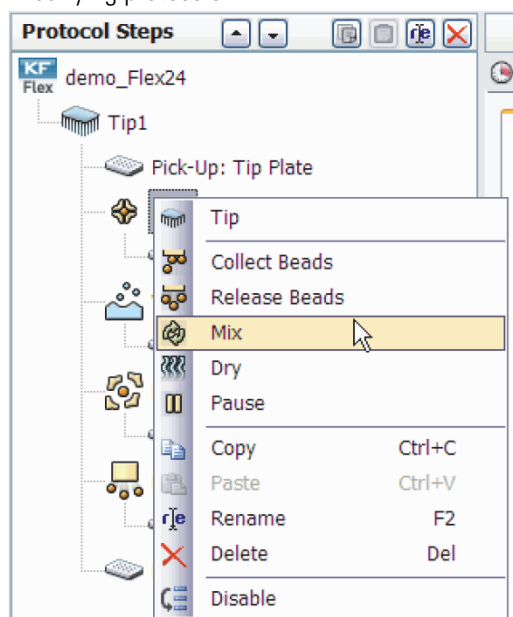
Modify Protocols

You can modify protocols by adding, deleting and reordering steps.

The command bar above the *Protocol Steps* tree provides you with controls for moving steps up and down, and copying, pasting, renaming and deleting steps.

You can also access the context menu by right-clicking in the *Protocol Steps* tree. In addition to the controls in the command bar. The context menu also allows you to add steps and disable existing steps.

Figure 16. Modifying protocols



To edit a protocol:

1. Select a protocol step from the *Protocol Steps* tree.
2. Select the appropriate action from the action panel, command bar or context menu.

Note New protocol steps are added to the end of the protocol sequence. Use the up and down arrow controls in the command bar to reorder the steps.

For information on modifying individual step parameters, refer to “[Protocol Steps and Parameters](#)” on [page 49](#).

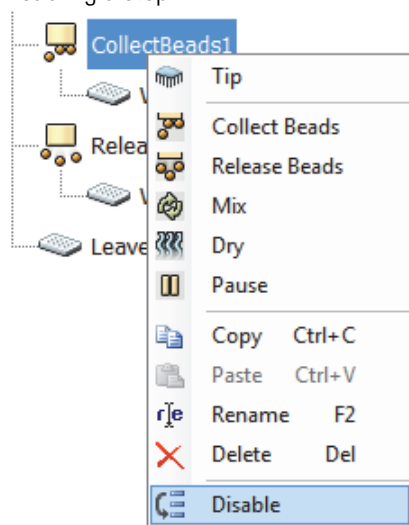
Disable or Enable Steps

You can disable steps before you execute a protocol. When you disable a step and execute the protocol, the run skips the disabled step.

To disable or re-enable a step:

In the **Protocol** view, right-click the desired step in the *Protocol Steps* tree and select **Disable/Enable** from the menu.

Figure 17. Disabling a step



The step icon turns gray indicating that the step is disabled.

Calculate the Duration of a Step or Protocol

You can calculate the estimated duration of the entire protocol or an individual step before running the protocol. In the *Protocol Steps* tree view, click the name of the protocol or step, and click the **Calculate Duration** button.

The result is presented beside the **Calculate Duration** button.

If you are calculating the durations for KingFisher Presto, the calculation requires connection either to the instrument or the simulator. With other instrument types, no connections are necessary.

Save Protocols

You can save the protocol at any stage. You cannot run a protocol before you have saved it.

1. Click **Save**.
2. Select a location for the protocol.
3. Name the protocol.
4. Click **OK**.

To save the protocol with another name, click **Save As** in the **Home** view.

Note To edit protocols created with KingFisher Software 2.6.2, open the protocol and save it by using the **Save As** function. This saves the protocol as a BindIt protocol, after which you are able to edit the protocol freely. In this new BindIt protocol all speed parameters are set to medium speed. See [Table 7](#) for the mixing speeds.

Lock Protocols

Protect protocols by locking them. To lock a protocol in BindIt 4.0, select **Lock** either from the **Protocol** or the **Layout** view.

Once a protocol has been locked, it cannot be modified. The lock also cannot be removed. To open a locked protocol for editing, it must be saved with a new name (with Save As...) to create a new protocol for editing.

Delete Protocols

Delete protocols and run reports from the file system of your PC.

Delete a protocol from the instrument memory from the **Protocols** tab in the *Data Transfer* dialog.

Note You cannot delete factory or maintenance protocols from the instrument memory.

1. Check that the instrument has been configured correctly. See “[Define a New Instrument](#)” on [page 8](#).
2. In the **Home** view, click **Transfer**.
3. In the *Data Transfer* dialog, select the **Protocols** tab.
4. Select the protocol from the *Instrument protocols* list.

Note With KingFisher/MagMAX Express and KingFisher mL/BeadRetriever, check the protocol name from the instrument (when it is not connected) and enter the exact name into the Protocol name field.

5. Click **Delete**.
6. Click **Yes** to confirm the deletion.

Plate Change Logic

The KingFisher Flex and KingFisher 96/MagMAX Express 96 and the turntable have eight plate stations, but a protocol for these instruments can contain more than eight plates.

Before executing the protocol, note the following rules:

- The instrument prompts you to load the plate from which it will pick up the tips.
- The plate that is used in the first step is the last one to be loaded onto the turntable at the beginning of protocol execution.
- At the end of the run, the plate of the last step is handed out first.
- If more than eight different plates are used in the protocol, the tip comb is picked up before the other plates are required.

Execute the Protocol

Before you can execute protocols in BindIt Software, ensure that the required instrument has been defined in the software settings. For more information, see “[Define a New Instrument](#)” on [page 8](#).

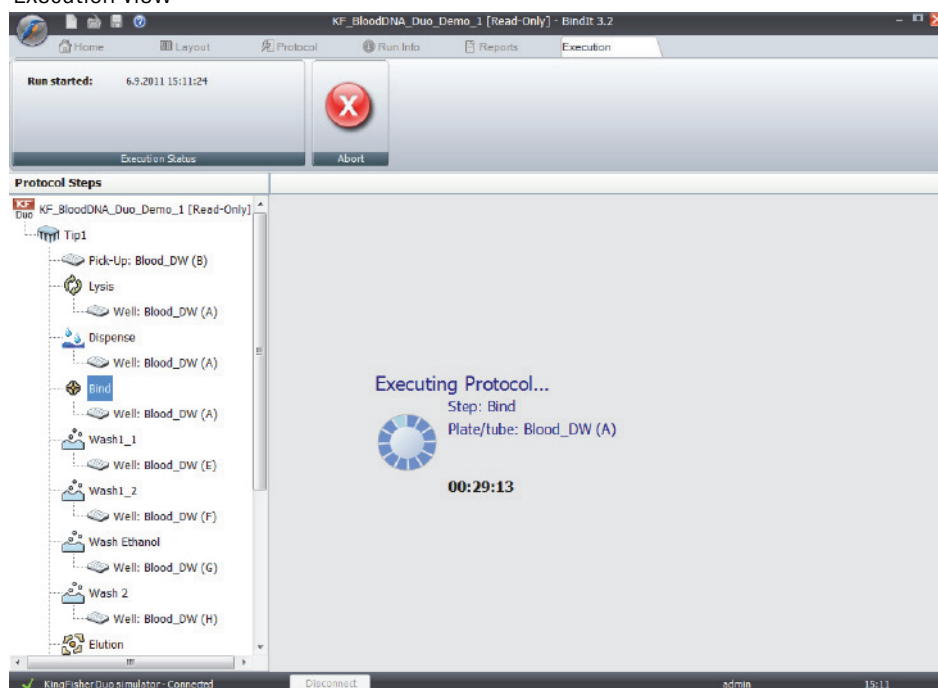
Note You can run a protocol from the instrument after you have transferred the protocol to the instrument.

To execute a protocol:

1. Click **Start**.
2. Enter a name for the protocol.
3. Click **OK**.

BindIt Software starts running the protocol steps and the **Execution** view opens.

Figure 18. Execution view



Follow the instructions on the instrument display to complete the protocol steps.

You can start entering [Add Run Information](#) when the protocol execution has begun.

To stop the process at any time, click **Abort**. If you have aborted the current run by pressing the **Stop** button on the instrument, you also have to click **Abort** in BindIt Software.

When the protocol is executed, the **Reports** view opens showing the status report of the protocol run.

Add Run Information

Run information can be entered before, during or after protocol execution, but it is typically entered when the protocol execution has been started.

This feature is not available for KingFisher Presto.

Add Sample IDs

To enter sample IDs:

1. Go to the **Sample** tab.
2. Write, paste or use a barcode reader to read the identifiers into the *Sample ID* field.
3. Set the fill direction.
4. Enter the number of replicates and their direction.
5. Select the starting point in the layout.
6. Click **Add**.

You can also read sample IDs directly from a text file (.txt) in which they must be on separate lines. To read a text file into the *Sample ID* field, click the arrow next to the field name.

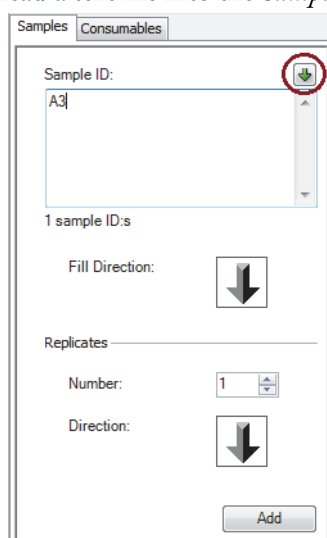


Figure 19. Sample ID

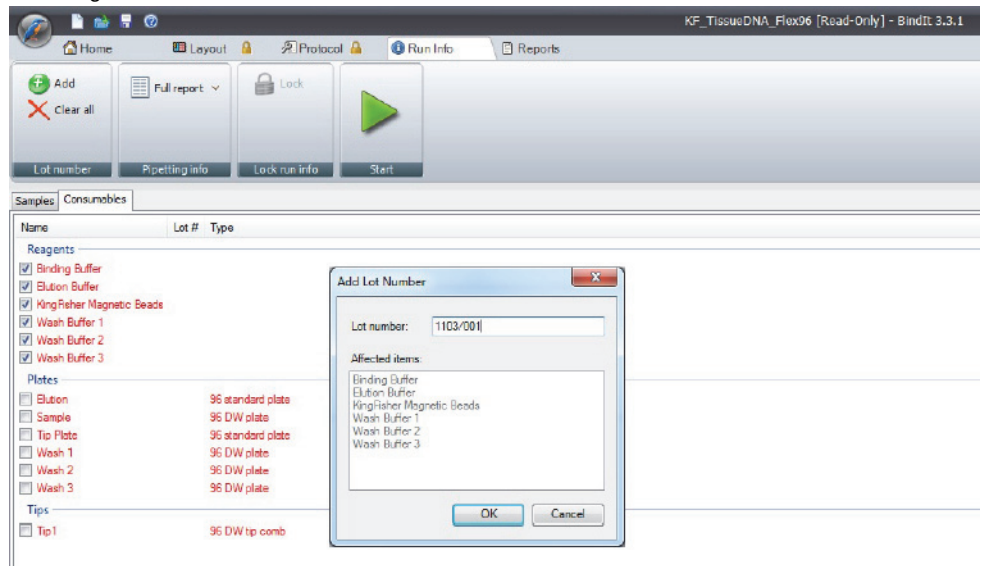
You can also add sample IDs directly into the cells of the layout grid by clicking a cell and entering the data. If you use the same identifier for two or more cells, they are automatically marked as replicates.

Add Lot Information

To enter lot information:

1. Go to the **Consumables** tab.
2. Tick the desired reagents.
3. Click **Add**.
4. Enter the lot number.

Figure 20. Entering the lot number



5. Click **OK**.

Lock Run Information

You can protect any entered run-specific information by clicking **Lock** in the **Run Info** view.

Locking run information before execution automatically saves the run as a locked and non-executed run. Run information can also be locked after execution.

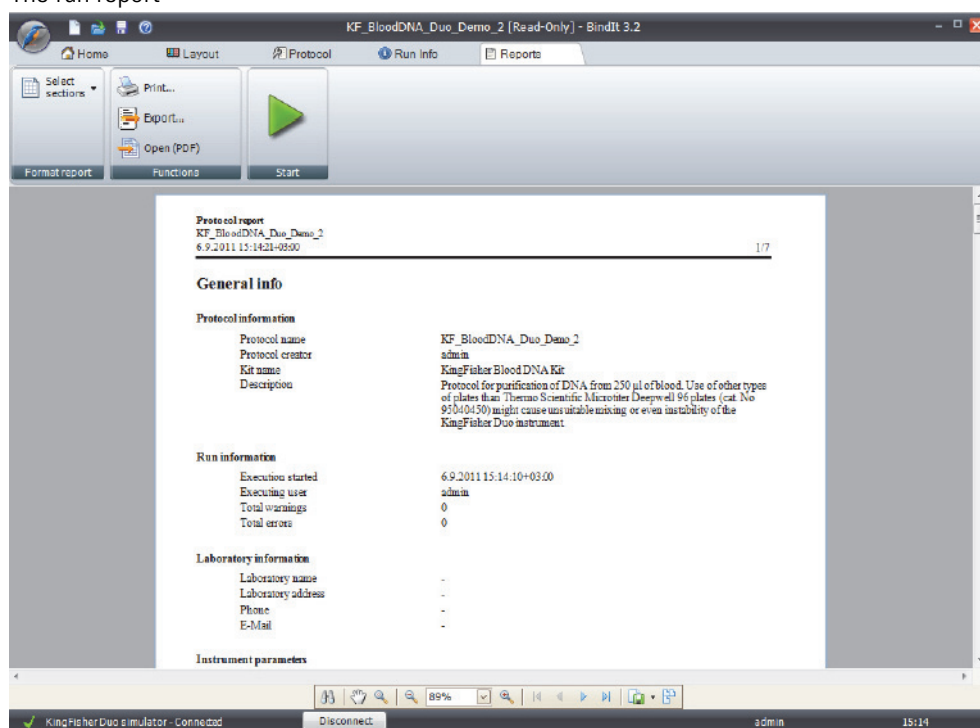
Note Once run information is locked, the operation cannot be undone and the information cannot be altered.

View the Run Report

The **Reports** view shows the protocol run report.

After the protocol has been executed, you can view the run log. The report also contains information on individual steps and the plate layout.

Figure 21. The run report



From the drop-down menu under **Select selections**, select which sections of the report you want to see.

To print the report, click **Print**.

To export the report, click **Export**. You can save the exported report in **.xlsx**, **.rtf**, or **.pdf** format.

To open the report as a PDF file, click **Open (PDF)**.

Export Results

To export results:

1. Click **Export** in the **Reports** view.
2. Name the file.
3. Select the saving format (an Excel workbook, a text file or a PDF).
4. Click **Save**.

Transfer Protocols

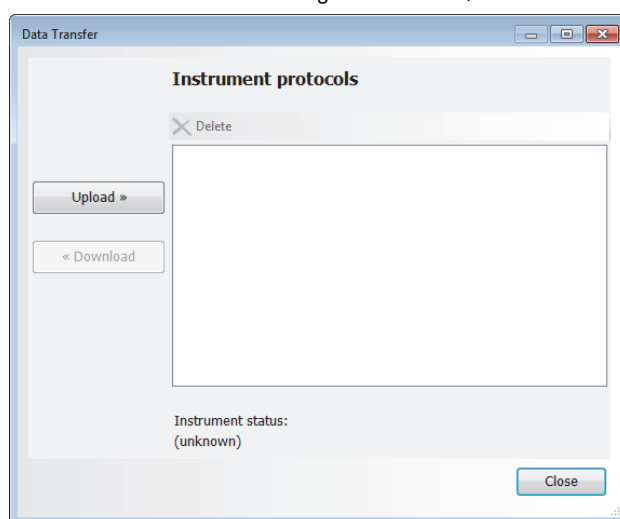
You can upload your protocols from your PC to the instrument. Depending on the instrument you can also download the instrument protocols back to your PC or create backups of them to your PC.

Note The acceptable characters for instrument protocol names are letters, numbers, dashes and underscores.

Upload Protocols to the Instrument

You can upload a protocol from your PC to the instrument memory, after which you can run the protocol from the instrument. From below, select the correct set of instructions based on the instrument you are using.

Figure 22. Protocol transfer view in KingFisher Presto, the view varies depending on the instrument



❖ Upload a protocol to KingFisher Presto

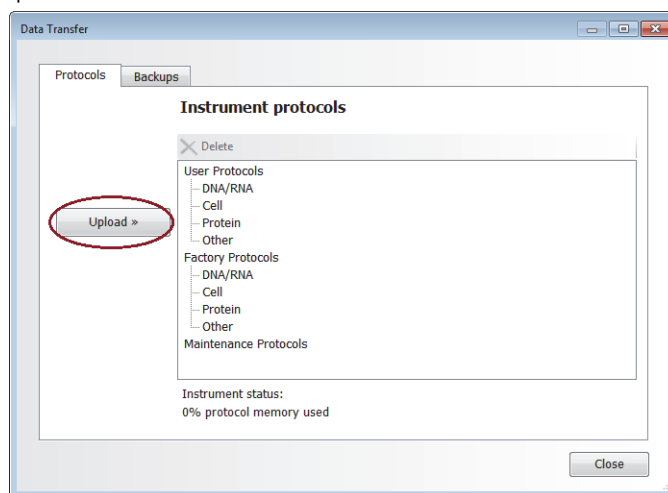
1. Check that the instrument is connected to the PC.
2. In the **Home** view, click **Transfer**.
3. Click **Upload**.
The file system opens.
4. Find the protocol you want to transfer and click **Open**.

Note The transfer of a protocol may take a while depending on the size of the protocol.

❖ Upload a protocol to KingFisher Duo Prime/Duo or KingFisher Flex

1. Check that the instrument is connected to the PC.
2. In the **Home** view, click **Transfer**.
3. Click **Upload**.

Figure 23. The Upload button



The file system opens.

4. Find the protocol you want to transfer and click **Open**.
5. If you are using KingFisher Flex, edit the name of the protocol if necessary.
6. Select the protocol category from the drop down list.

Your protocols are transferred to the *User Protocols* category. Factory protocols are automatically transferred to *Factory Protocols* category.

Note You cannot transfer a protocol that has the same name as one already in the instrument memory.

7. Click **OK**.

Note The transfer of a protocol may take a while depending on the size of the protocol.

8. After the transfer has been completed, click **OK**.

The transferred protocol appears in the *Instrument protocols* field and you can launch it in the instrument.

Note If the transfer does not succeed, check the status of the instrument memory. If the protocol memory is full, remove protocols from the instrument memory. See "[Delete Protocols](#)" on [page 37](#).

❖ Upload a protocol to KingFisher 96

1. Check that the instrument is connected to the PC.

2. In the **Home** view, click **Transfer**.

3. Click **Upload**.

The file system opens.

4. Find the protocol you want to transfer and click **Open**.

5. Change the protocol name if necessary.

Note You cannot transfer a protocol that has the same name as one already in the instrument memory.

6. Click **OK**.

Note The transfer of a protocol may take a while depending on the size of the protocol.

7. After the transfer has been completed, click **OK**.

The transferred protocol appears in the *Instrument protocols* field and you can launch it in the instrument.

Note If the transfer does not succeed, check the status of the instrument memory. If the protocol memory is full, remove protocols from the instrument memory. See “Delete Protocols” on page 37.

❖ **Upload a protocol to KingFisher or KingFisher mL**

1. Check that the instrument is connected to the PC.

2. In the **Home** view, click **Transfer**.

3. Type a name for the protocol.

4. Click **Upload**.

The file system opens.

5. Find the protocol you want to transfer and click **Open**.

6. Click **Yes**.

Note The transfer of a protocol may take a while depending on the size of the protocol.

7. When the transfer is complete, click **OK**.

Download Protocols from the Instrument

You can only download protocols from the instrument back to your PC with KingFisher Presto and King Fisher Duo Prime/Duo. These protocols remain usable both on the instrument and back on the PC. This function can be used to create backups of instrument protocols with these instruments.

To download protocols:

1. Check that the instrument is connected to the PC.

2. In the **Home** view, click **Transfer**.

3. Select the protocol from the list.
4. Edit the protocol name if necessary.
5. Click **Save**.
6. Click **Download**.

The protocol is transferred back to the file system of your PC.

Backups

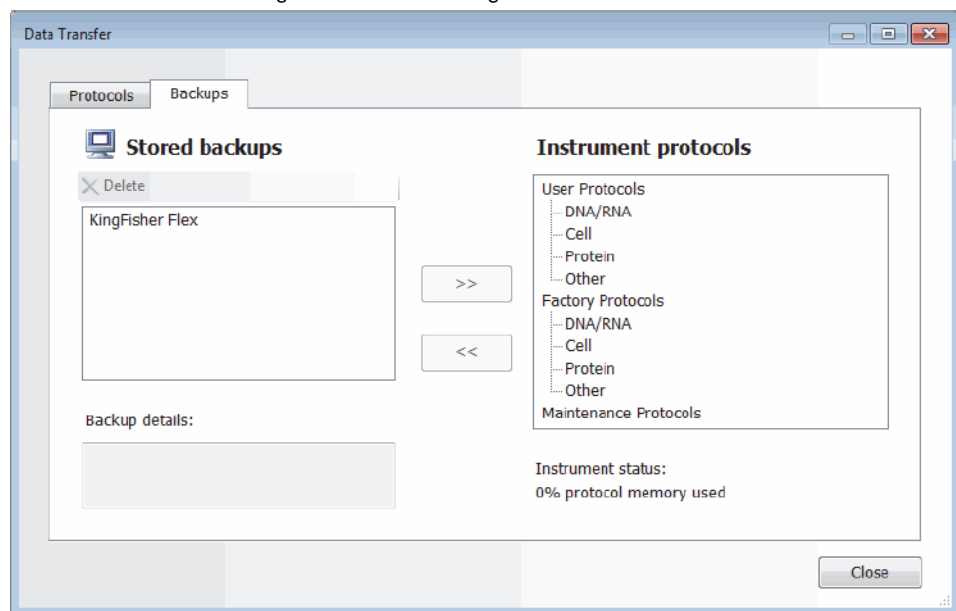
Backups are copies of the protocols you have uploaded to the instrument memory. Backups cannot be opened or edited in BindIt Software but they can be transferred back to the instrument or to other instruments anytime.

You can only create protocol backups with KingFisher Flex, KingFisher, KingFisher mL and KingFisher 96.

You can create backups of the protocols in the instrument memory and transfer them to the file system of your PC from the **Backups** tab in the *Data transfer* dialog.

1. In the **Home** view, click **Transfer**.
2. Click the **Backups** tab.
3. Select the protocol in the *Instrument protocols* list.

Figure 24. Data transfer view in KingFisher Flex and KingFisher mL



4. Click the button with the arrow pointing left.
5. Change the name of the protocol, if necessary, and click **OK**.

The protocol is transferred to the file system of the PC, and appears in the *Stored backups* field after the transfer has been completed.

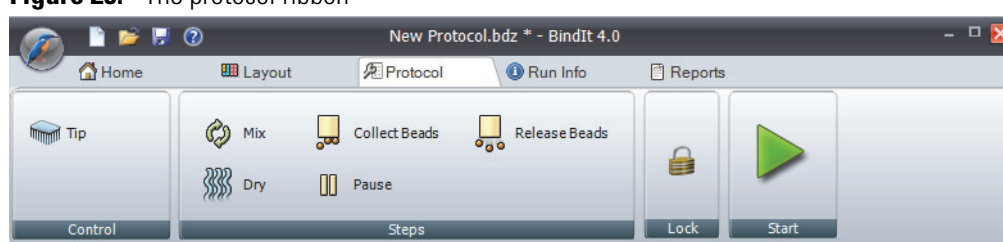
6. Click **OK**.

To transfer a protocol backup to the instrument, select the protocol from the *Stored backups* list and click the button with the arrow pointing right.

Protocol Steps and Parameters

This chapter describes the different steps and parameters in the Protocol view.

Figure 25. The protocol ribbon



Tip

A *Tip* step is automatically added to the step tree when the protocol is created.

You can add as many tip combs to the protocol as necessary. For more information on tip features, operations and positions, refer to “[Tip Operation](#)” on [page 21](#).

Pick-Up and Leave

Pick-Up and *Leave* are fixed steps at the beginning and end of every protocol in KingFisher Presto, KingFisher Duo Prime/Duo, KingFisher Flex and KingFisher 96.

In the *Pick-Up* step you select the plate or plate row from which the tip comb is picked up at the beginning of the protocol.

In the *Leave* step you select the plate or plate row to which the tips are left at the end of the protocol.

The steps have the following parameters:

- **Plate.** Select the used plate type.
- **Well.** Select the location from where the tip comb is picked or where it will be left at the end of the protocol execution.

Collect Beads

The *Collect Beads* step is used for collecting the magnetic beads from a specified well/tube. This step can be repeated as many times as necessary.

The step has the following parameters:

- **Collect count.** The number of times the tip moves with the magnetic rod in the reagent in order to collect all the beads. A default value is set automatically.
- **Collect time.** The duration of the collecting action at the bottom of the well at each collection.

Release Beads

The *Release Beads* step is used to release the collected magnetic beads to a specified well/tube. If you do not add this step, the magnetic particles remain attached to the tip.

The step has the following parameters:

- **Release time (hh:mm:ss).** The time that the tip shakes in the reagent in order to release all the beads. A default value is set automatically.
- **Speed.** The speed of the mixing action during Release time. The default speed is Fast. The available speeds are listed in “Tip speeds” on page 75.

Mix

The *Mix* step is used for various functions. In the *Mix* step, you can define parameters for mixing, heating, binding, washing and eluting the reagents. You can also change the step icon to match the functions you are using.

The *Mix* step has the following parameters:

- Beginning of step
- Step icon
- Mixing/Pause or Mixing/Heating depending on the instrument
- End of step

To see all parameter value options by clicking **Show Advanced**. To hide some of the fields and return the parameters to the default values, click **Reset Advanced**.

Beginning of Step

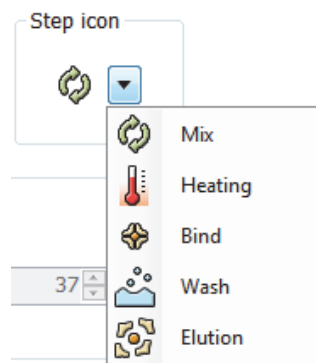
The *Beginning of step* field includes the following parameters:

- **Precollect.** If selected, the magnetic rods go to the bottom of the tip, the tip comb and the magnetic rods go to the bottom of the well, and collect the particles.
- **Release beads [hh:mm:ss].** If selected, the beads are released into the reagent at the beginning when there are no beads in the reagent. Enter the time that the tip shakes in the reagent in order to release all the beads.
- **Speed.** The shaking speed during the Release beads step. The default speed is Fast. The available speeds are listed in “Tip Speeds” on page 23.

Step Icon

You can change the step icon in the *Protocol Steps* tree by selecting the *Mix*, *Heating*, *Bind*, *Wash* or *Elution* icon from the list.

Figure 26. Step icon



Note Selecting the icon has purely an illustrative effect. The step functions according to the given parameter values.

Mixing/Pause Parameters

The *Mixing/Pause parameters* field is included in KingFisher/MagMAX Express and KingFisher mL/BeadRetriever.

The *Mixing/Pause parameters* field includes the following parameters:

- **Pause for manual handling.** If selected, the protocol is stopped, for example, to transfer the strip to a heating block.
- **Message.** Enter a message to be displayed by the instrument during the pause.
- **Mix time [hh:mm:ss].** The time that the tip shakes in the reagent in order to mix the reagent with or without the beads. A default value is set automatically. You can specify times for up to three different mixing periods.
- **Speed.** The mixing speed. The default speed is *Medium*. Other available speeds are *Slow*, *Fast*, *Bottom mix* and *Half mix* (see “[Tip Speeds](#)” on [page 23](#)).

You can also select *Paused* as the speed. If selected, the processing stops for the time defined in **Mix time [hh:mm:ss]**. You can choose any of the following tip positions.

The *Tip position when paused* field has the following options:

- **Outside well/tube.** The plastic tips are outside the well or tube.
- **Above well/tube surface.** The plastic tips are at the well or tube surface but do not touch the liquid. This is the default position.
- **Tip edge in liquid.** The plastic tip edge is in the liquid.

See [Figure 9](#) for the different tip positions.

- **Loop count.** The number of times the mixing sequence is carried out.

Mixing/Heating Parameters

The *Mixing/Heating parameters* field is included in KingFisher Presto, KingFisher Duo Prime/KingFisher Duo, KingFisher Flex and KingFisher 96/MagMAX Express 96.

The *Mixing/Heating parameters* field includes the following parameters:

- **Mix time [hh:mm:ss]**. The time that the tip shakes in the reagent in order to mix the reagent with or without the beads. A default value is set automatically. You can specify times for up to three different mixing periods.
- **Speed**. The mixing speed. The default speed is *Medium*. Other available speeds are *Slow*, *Fast*, *Bottom mix* and *Half mix* (see “Tip Speeds” on page 23).

You can also select *Paused* as the speed. If selected, the processing stops for the time defined in **Mix time [hh:mm:ss]**.

In KingFisher Presto, KingFisher Flex and KingFisher 96/MagMAX Express 96, it is possible to heat the plates without mixing the liquid. In KingFisher Duo Prime/KingFisher Duo, heating is possible in plate row A and the elution strip.

You can then choose the different tip position for the heating action.

The *Tip position when paused* field has the following options:

- **Outside well/tube**. The plastic tips are outside the well or tube.
- **Above well/tube surface**. The plastic tips are at the well or tube surface but do not touch the liquid. This is the default position.
- **Tip edge in liquid**. The plastic tip edge is in the liquid.

See [Figure 9](#) for the different tip positions.

- **Loop count**. The number of times the mixing sequence is carried out.
- **Heating during mixing**. Tick this box if you want the reagents to be heated during the step.

Note With KingFisher Presto, KingFisher Flex and KingFisher 96/MagMAX Express 96, depending on the heated plate, you need to have the correct heating block inserted. All plates, including Deep well, 96 standard and PCR plates, can be heated using the compatible heating blocks. Refer to the instrument user manuals for information on the correct heating blocks for each plate type.

- **Preheat**. (Except KingFisher Duo Prime/Duo) If selected, the heating block is preheated during the execution of the previous steps in the protocol before it starts to heat the plate. The plate is heated to the set temperature during the period defined in **Mix time [hh:mm:ss]**. If **Preheat** is not selected, the heating block is cold when **Heating during mixing** starts, and the block is heated up during the **Mix time** period.

Note With KingFisher Presto, KingFisher Flex and KingFisher 96/MagMAX Express 96, if you have selected **Heating during mixing** in the first *Mix* step in the protocol and you have also chosen **Preheat**, the heating block does not have enough time to warm up to a high temperature. The heater can heat the heating block approximately 10°C per minute

- **Block temperature** [°C]. The temperature to which the heating block is heated during the time defined in **Mix time** [hh:mm:ss]. The default value is 50°C. With KingFisher Duo Prime/Duo, the heating and elution block temperature is from +10°C to 75°C (heating block) and from +4°C to 75°C (elution block) when instrument is at room temperature. With KingFisher Presto and KingFisher Flex, the block temperature is from +5°C above ambient temperature to +115°C, and with KingFisher 96/MagMAX Express 96, from +5°C above ambient temperature to +96°C.



CAUTION The material of the plate may melt and stick to the heating block, if the temperature is above 90°C.

Note In KingFisher Presto, KingFisher Duo Prime/Duo and KingFisher Flex, **Block temperature** [°C] shows the temperature of the heating block, and in KingFisher 96/MagMAX Express 96, it shows the temperature of the liquid.

Note In KingFisher 96/MagMAX Express 96, the actual heating block temperature is higher than the set temperature to ensure that the liquid reaches and maintains the desired temperature.

Note The higher the set temperature and the greater the liquid volume, the longer it takes for the liquid to reach the set temperature.

End of Step

The *End of step* field includes the following parameters:

- **Postmix** [hh:mm:ss]. If selected, the tip (without the magnetic rod) mixes the reagent. The parameter sets the mixing period.
 - **Speed**. The mixing speed during Postmix. The default speed is Medium.
- **Collect beads count** (1...5). Select this if you want the beads to be collected from the reagent and transferred to the next plate or well. By default, the beads are collected. Select the number of times the tip moves with the magnetic rod in the reagent in order to collect all the beads. A default value (3) is set automatically.
 - **Collect time** (0...30 s). The duration of collecting beads in seconds.
- **Post-temperature** [°C] (**heating/cooling**). (KingFisher Duo Prime/Duo only) When selected, the heating block retains the defined temperature for steps following the *Mix* step until either another *Mix* step changes the temperature or the protocol ends.

Dry

The *Dry* step dries the magnetic beads above or inside a specific well or tube. During the *Dry* step the beads remain attached to the tip surface, and the magnetic rod remains inside the tip.

The beads can be dried inside the well or tube above the liquid level or with the tip completely lifted from the well or tube. This step can be repeated as many times as necessary.

The *Dry* step has the following parameters:

- **Dry time** [hh:mm:ss]. The duration of the drying time. A default value is set automatically.

- **Tip position.** The position of the tips.
 - **Outside well/tube.** The plastic tips are outside the well or tube.
 - **Above well/tube surface.** The plastic tips are at the well or tube surface but do not touch the liquid. This is the default position.

Pause

The *Pause* step is used to pause protocol execution. During the pause, you can, for example, dispense new reagents.

The plastic tips are raised to the highest position with the *Pause* step. In the step parameters, you can add a message to show during the pause. In KingFisher Presto, the possible message is displayed on the software whereas in other instruments it shows on the instrument display. The instrument continues with the protocol after you have pressed **Start** on the instrument keypad. This step can be repeated as many times as necessary.

The *Pause* step includes the following parameters:

- **Message.** Type the message that is shown in the *Execution Progress* dialog during the *Pause* step.
- **Dispense.** Select the checkbox for dispensing a new reagent during the protocol. This setting is required with KingFisher Presto, KingFisher Duo Prime/ Duo, KingFisher Flex, and KingFisher 96/MagMAX Express 96, as it instructs the instrument to move the plate to the loading position for user actions.

When selected, you can define the parameter values in the *Dispensing parameters* field.

- **Volume [µl].** Enter the correct volume for the reagent in the field.
- **Reagent name.** Type the reagent name in the field.

Settings

To access BindIt Software settings, click the **Settings** icon in the **Home** view.

The **Settings** menu is divided into two categories:

- Options with software settings.
- Instruments with instrument related settings.

Options

In the **Home** view, click **Settings** to edit or view the software options.

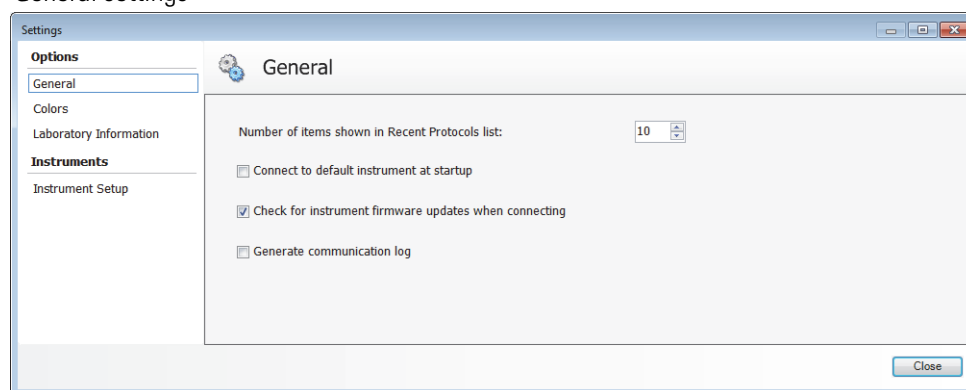
The Options menu includes three different dialogs:

- General
- Colors
- Laboratory Information

General

The *General* dialog under **Settings** includes settings related to default connection and locking the software.

Figure 27. General settings



The *General* dialog includes the following parameters:

- **Number of items shown in the Recent Protocols list.** This selection determines the number of items displayed in the **Recent Protocols** list in the **Home** view.

- **Connect to default instrument at startup.** When selected, the software connects to the default instrument at startup. When you create the default instrument for the first time, the option is enabled. Otherwise, the instrument is not connected and the user has to establish the connection manually.

For setting a default instrument, see “[Instruments](#)” on page 56.

- **Check for instrument firmware updates when connecting.** This feature checks for available instrument firmware updates when an instrument is connected to BindIt Software. If a new firmware is available for the connected instrument, the user is prompted to update. If accepted, BindIt Software disconnects from the instrument and starts a separate, instrument-specific firmware update procedure. Follow the instructions presented on screen to complete the update procedure.
- **Generate communication log.** This feature should be used only for troubleshooting purposes, for example, if instructed by technical support. When enabled, BindIt Software generates a communication log file (My Documents\BindIt\Communication.log). Do not leave the feature on for prolonged periods to prevent the log file from growing.

Colors

In the *Colors* dialog under **Settings** you can select the color theme of the user interface. The color of the user interface changes after you close the dialog.

Laboratory Information

You can give information on the laboratory performing the protocols in the **Laboratory Information** dialog under **Settings**. This information will be displayed in the reports.

Type the information in the following fields of the *Laboratory Information* dialog:

- Laboratory name
- Address
- Telephone
- E-mail

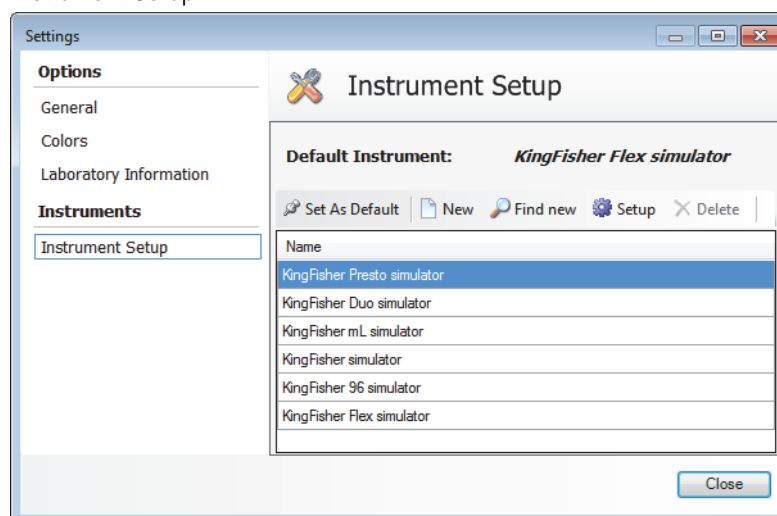
Instruments

Select **Instrument Setup** under **Settings** to open the *Instrument Setup* dialog.

Instrument Setup

In the *Instrument Setup* dialog, you can add, edit and delete instruments from the software. You can also create an instrument report of the selected instrument.

Figure 28. Instrument Setup.



The *Instrument Setup* dialog includes the following functions:

- **Set As Default** sets the selected instrument as the default instrument. The software connects to the default instrument at startup if **Connect to default instrument at startup** is selected in **General** under **Options**.
- **New** opens the *New Instrument* dialog in which you can define a new instrument you want to connect to the software.

The *New Instrument* dialog includes the **General** and **Communications** tabs.

- **Find new** searches for new USB connected KingFisher instruments when clicked
- **Setup** opens the *Instrument Setup* dialog for editing the setup parameters of an existing instrument.

The *Instrument Setup* dialog includes the **General** and **Communications** tabs.

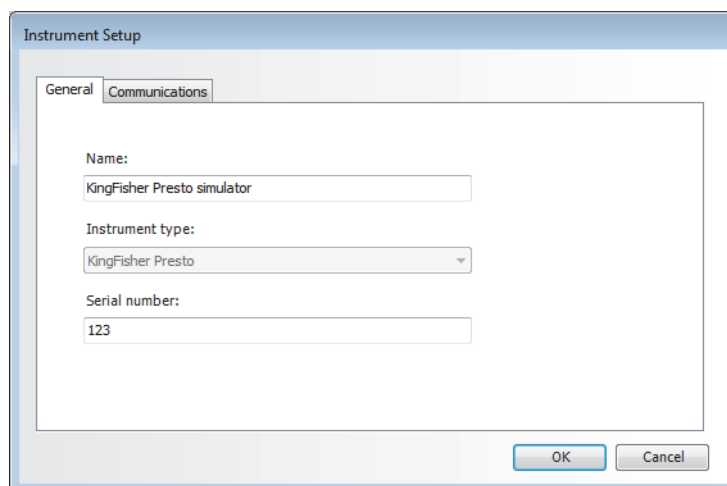
- **Delete** removes the selected instrument from the software.

Note You cannot delete the default instrument from the software. If you want to delete the instrument that has been selected as the default instrument, first set another instrument as the default instrument.

General

Click **New** or **Setup** to add or check the general information related to the instrument on the **General** tab.

Figure 29. The General tab



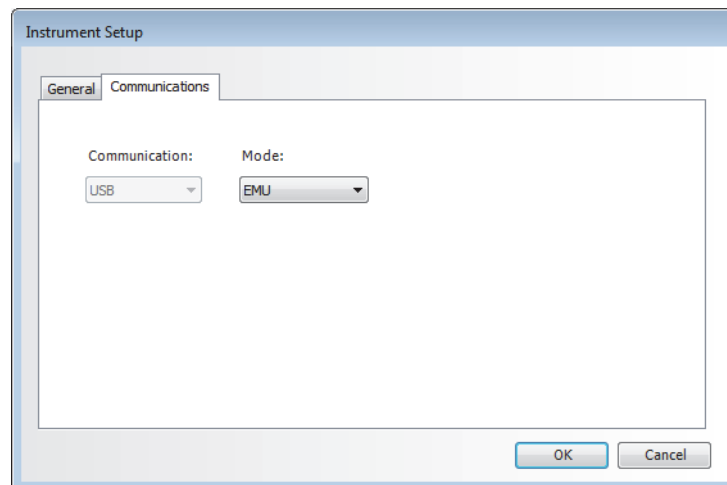
The **General** tab has the following parameters:

- **Name.** The unique name of the specific instrument.
- **Instrument type.** The type of the instrument.
- **Serial number.** Check the serial number of the instrument from the type label on the instrument and enter it here.

Communications

After clicking **New** or **Setup**, define the communication settings between the instrument and the PC on the **Communications** tab.

Figure 30. The Communications tab



The **Communications** tab includes the following parameters:

- **Communication.** Select the type of data communication port. The available port types are COM and USB, depending on the instrument model.
- **Mode.** Select EMU for the simulator mode and depending on the cable you are using, select COM or USB for the instrument mode.

- **Port number.** (Serial COM only) Select the COM port that the instrument is connected to. Allowed port numbers are 1 to 100.
- **Speed.** (Serial COM only) The speed is set to 9,600 or 19,200.

Automation Interface

In an automation environment, BindIt Software can be controlled through an automation interface, which allows the automation client to run protocols created with BindIt Software. Only KingFisher Presto and KingFisher Presto can be used in an automation environment.

If you are using KingFisher Presto, refer to *Thermo Scientific™ KingFisher™ Presto Integration guide* (Cat. no. N17647) for more information. If you are using KingFisher Flex, refer to the *Thermo Scientific BindIt Automation Interface User Manual* (Cat. no. N17648). Both of these manuals are on the BindIt Software installation CD provided with the instrument.

Help

To open BindIt Software online help, do one of the following:

- Click the **Help** button in the menu bar.
- Click the **Help** button in the **Home** view action panel.
- Press the **F1** key on your keyboard.

The Help toolbar buttons are:

- **Hide.** Hides the left-hand side navigation pane. To display the navigation pane again, click the Show button that appears instead of the Hide button.
- **Back.** Takes you back to the previous view in your view history.
- **Forward.** Takes you to the next view in your view history.
- **Print.** Prints a single topic or multiple topics.

Access the help content by selecting one of the following tabs:

- **Contents.** Browse the help topics by subject.
- **Search.** Find a specific help topic by entering words to search in the help content.

Troubleshooting

Note Do not use the instrument if it appears to malfunction.

When the system detects an error, the current operation is terminated. If an error occurs, it is best to abort the current run and restart from the beginning after the problem is solved.

The BindIt Software run report shows the error and warning messages that have occurred during a protocol run.

Note Some RS-232 serial communication adapters may cause connection problems. We recommend using a direct computer-to-instrument connection, when possible.

See below for possible solutions to issues that you may encounter while using BindIt Software.

Table 9. Troubleshooting

Problem	Solution
An instrument connection cannot be established	<p>On the instrument, check that the USB or serial cable is correctly set and securely connected. See the instrument's user manual for more information.</p> <p>Check that the communication settings match the communication interface in Settings > Instrument Setup.</p> <p>Switch the instrument OFF and ON again.</p>
Failed protocol transfer due to insufficient instrument memory	Delete unused protocols from the instrument memory to make room for new protocols.
The turntable does not turn during the dispense step (in KingFisher Presto, KingFisher Duo Prime/ Duo, KingFisher Flex or KingFisher 96/MagMAX Express 96)	Check that the <i>Dispense</i> box is ticked in the <i>Pause</i> step.
Inefficient magnetic bead transfer	<p>Increase the parameter values of the <i>Collect Beads</i> step.</p> <p>Add a specific <i>Collect Beads</i> step.</p>

Table 9. Troubleshooting

Problem	Solution
Inefficient washes	<p>Check that the beads are released at the beginning of the step.</p> <hr/> <p>When a new BindIt protocol is run for the first time, monitor the protocol to verify that the magnetic beads are moving in the liquid and not forming a clump. Increase the mixing speed or loop slower and faster mixing speeds to enhance the movement of the magnetic beads.</p>
Loss of the elution buffer after a run	<p>Check the parameters of the elution step. Long heated steps with fast mixing can increase evaporation and cause loss of the elution buffer.</p>
Tip plate or strip loading failure (in KingFisher Presto, KingFisher Duo Prime/Duo, KingFisher Flex or KingFisher 96/MagMAX Express 96)	<p>Check that the tip plate or strip is on the plate or row indicated in the BindIt protocol.</p>
Unable to modify locked protocol or run-specific information	<p>Save a copy of the protocol or run information with a new name to open it for editing.</p>

Example Protocol: KingFisher Duo Prime/Duo

This example helps you get acquainted with creating protocols and plate layouts for KingFisher Duo Prime/Duo.

We recommend that you create the appropriate example protocol, as this will help you to become acquainted with the use of the software. This is an example of DNA isolation from tissue lysate.

Creating the Plate Layout

1. Start BindIt Software.
2. In the **Home** view, click **New** and select **KingFisher Duo** from the drop-down menu.
3. In the **Layout** view, click **New** and select *96 DW plate* as the plate type.
4. Type *Tissue DNA* in the *Plate/Strip name* field.
5. To add an elution strip, click **New** and select *KingFisher Duo elution strip*.
6. Name the elution strip *Elution* in the *Plate/Strip name* field.

Proceed with entering reagent information.

Entering Reagent Information

Enter the reagent information for each well in the plate layout that you just created. Each well icon represents one row on the plate.

1. Select *Well A* in the tree view and name it *Sample* in the *Well name* field. Note that well names are optional.
2. Enter the reagent information for *Well A* (Sample).






CAUTION Make sure the reagent volume does not exceed the volume range given in [Table 8](#).

Figure 31. Entering reagent information to *Well A*.

Reagent information

✖ Delete | Total volume: Maximum:


Reagent name	Volume [ul]	Color	Type
Lysed sample	225		Reagent
KingFisher Magnetic Beads	25		Reagent
Binding Buffer	360		Reagent
Type a name to add new...			

3. Select *Well B* and name it *Tip comb*.
4. Select *Well F*, name it *Wash 1* and enter the reagent information.

Figure 32. Entering reagent information to *Well F*.

Reagent information

✖ Delete | Total volume: Maximum:


Reagent name	Volume [ul]	Color	Type
Wash Buffer 1	600		Reagent
Type a name to add new...			

5. Select *Well G*, name it *Wash 2* and enter the reagent information.

Figure 33. Entering reagent information to *Well G*.

Reagent information

✖ Delete | Total volume: Maximum:

Reagent name	Volume [ul]	Color	Type
Wash Buffer 2	600		Reagent
Type a name to add new...			

6. Select *Well H*, name it *Wash 3* and enter the reagent information.

Figure 34. Entering reagent information to *Well H*.

Reagent information

✖ Delete | Total volume: Maximum:

Reagent name	Volume [ul]	Color	Type
Wash Buffer 3	800	<input type="color" value="#0000FF"/>	Reagent
Type a name to add new...			

7. Select the *Elution* elution strip and enter the reagent information.

Figure 35. Entering reagent information to the elution strip.

Reagent information

✖ Delete | Total volume: Maximum:

Reagent name	Volume [ul]	Color	Type
Elution Buffer	100	<input type="color" value="#FFFF00"/>	Reagent
Type a name to add new...			

Proceed with entering general protocol data.

Entering General Protocol Data

1. Go to the **Protocol** view.
2. Optionally enter the *Kit name* and *Protocol description* information.
3. Click the **Tip** icon in the tree view.
4. Select *KingFisher Duo 12-tip comb* from the **Tip** menu.

Proceed with adding steps to the protocol.

Adding Steps to the Protocol

This example protocol has six steps as well as the tip *Pick-Up* and *Leave* steps. Add the steps to the protocol in the given order.

Tip When adding *Mix* steps, click the **Show Advanced** button to view all parameter fields.

The tip comb is picked up from and left to the *Tissue DNA* plate.

Click the **Pick-Up** step in the protocol tree and select *Tissue DNA* as the plate and *B (Tip comb)* as the well.

Click the **Leave** step and select *Tissue DNA* as the plate and *B (Tip comb)* as the well.

Adding the First Step (Bind)

1. Click the **Mix** button.
2. Rename the step as *Bind*.
You can rename all the steps in the protocol.
3. Change the step icon to *Bind*.
4. Enter the values as shown in the figure below.

Figure 36. Entering the values for *Bind*.

The screenshot shows the configuration for a 'Bind' step. It is organized into three sections:

- Beginning of step:**
 - Precollect
 - Release beads [hh:mm:ss]: 00:00:30
 - Speed: Bottom mix
- Mixing/Heating parameters:**
 - Mix time [hh:mm:ss]: 00:04:30
 - Speed: Half mix
 - Heating during mixing
 - Block temperature [°C]: 37
 - Loop count (1...100): 1
- End of step:**
 - Postmix
 - Collect beads, count (1...5): 3
 - Collect time (0...30 s): 1
 - Post-temperature (heating/cooling)

5. Click *Well* in the tree view and select the *Tissue DNA* plate in the *Plate/Strip* field.
6. Select *A* as the well for the step from the *Well* list.

Adding Washes

1. Add the second step:
 - a. Click the *Bind* step in the tree view.
 - b. Click the **Mix** button.
2. Rename the step as *Wash 1*.
3. Change the step icon to *Wash*.
4. Enter the values as shown in the figure below.

Figure 37. Entering the values for *Wash*.

Beginning of step

Precollect

Release beads [hh:mm:ss]: 00:00:10 Speed: Bottom mix

Step icon

Mixing/Heating parameters

Mix time [hh:mm:ss]: 00:01:00 Speed: Half mix Heating during mixing

00:00:00 (none) Block temperature [°C]: 37

00:00:00 (none)

Loop count (1...100): 1

End of step

Postmix

Collect beads, count (1...5): 3 Collect time (0...30 s): 1

Post-temperature (heating/cooling)

5. Click *Well* in the tree view and select the *Tissue DNA* plate in the *Plate/Strip* field.
6. Select *F (Wash 1)* as the well for the step.
7. Add another *Mix (Wash)* step to the protocol with the same values as the first *Wash 1* step.
8. Rename the step *Wash 2* and select *G* as the well for the step.
9. Add one more *Mix (Wash)* step to the protocol.
10. Rename the step *Wash 3* and select *H* as the well for the step.
11. Enter the step values as shown below.

Figure 38. Entering the values for *Wash. 3*

Beginning of step

Precollect

Release beads

Step icon

Mixing/Heating parameters

Mix time [hh:mm:ss]: 00:00:30 Speed: Slow

End of step

Collect beads

Adding the Elution Step (Mix)

1. Click the **Mix** button.
2. Rename the step as *Elution*.
3. Change the step icon to *Elution*.
4. Enter the step values as shown below.

Figure 39. Entering the values for *Elution*.

5. Click *Well* in the tree view and select the *Tissue DNA* plate in the *Plate/Strip* field.
6. Select the *Elution* strip in the *Plate/Strip* field.
7. Select *A* as the well for the step.

Adding Bead Release and Collection Steps

1. Click **Release Beads**.
2. Set the *Release time* and *Speed* values.

Figure 40. Entering *Release time* and *Speed*.

3. Click *Well* in the tree view and select the *Tissue DNA* plate in the *Plate/Strip* field.
4. Select *H (Wash 3)* as the well for the step.
5. Click **Collect Beads**.
6. Set the *Collect count* and *Collect time* values.

Figure 41. Entering *Collect count* and *Collect time*.

7. Click *Well* in the tree view and select the *Elution* strip in the *Plate/Strip* field.
8. Select *A* as the well for the step.
9. Click **Release Beads**.
10. Set the *Release time* and *Speed* values.

Figure 42. Entering *Release time* and *Speed*.

Release time [hh:mm:ss]:

Speed:

11. Click *Well* in the tree view and select the *Tissue DNA* plate in the *Plate/Strip* field.
12. Select *H (Wash 3)* as the well for the step.

Saving the Protocol

1. Click **Save**.
2. Select where you want to save the protocol.
3. Name the protocol.
4. Click **OK**.

Running the Protocol

Before running the protocol, you can enter run-specific information, such as sample identifiers and consumable lot information, in the **Run Info** view.

Note Verify the protocol with the instrument simulator before running it with an actual instrument.

To run the protocol:

1. Switch the instrument on.
2. Check that the instrument has been added to the PC, and that the instrument is connected to the USB port.

If the instrument has not been added to the PC, follow the instructions in “[Define a New Instrument](#)” on [page 8](#). For further information, refer also to the *Thermo Scientific™ KingFisher™ Duo User Manual*.
3. Click **Connect** at the bottom of the view.
4. Click **Start** to launch the protocol directly without transferring it to the instrument memory.
5. Name the .KFrunc file.
6. Follow the instructions on the instrument display to load the *Tissue DNA* plate including the tips and the elution strip.

After the protocol run has completed, BindIt Software displays the protocol report in the **Reports** view.

A Example Protocol: KingFisher Duo Prime/Duo

Example Protocol: KingFisher Flex

This example helps you to get acquainted with creating protocols and plate layouts for KingFisher Flex. This example is basically valid also for KingFisher Presto and KingFisher 96/MagMAX Express 96.

We recommend that you create this protocol by working through the example, as this will help you to become acquainted with BindIt Software.

This is an example of DNA isolation from a blood sample.

Creating the Plate Layout

1. Start BindIt Software.
2. In the **Home** view, click **New** and select *KingFisher Flex* from the drop-down menu.
3. Click **New** and select *96 DW plate* as the plate type.

Entering Reagent Information

When entering the reagent information, choose the colors for the different reagents to your own taste. The proportions of the reagents are shown according to the selected colors in the *Content information* field.




1. Name the first plate *Sample* in the *Plate name* field, and press **Enter**.
2. Enter the reagent information as shown in the following figure.



CAUTION Make sure that the reagent volume is within the limits given in [Table 8](#).

Figure 43. Entering reagent information to *Sample*


Reagent information

Delete		Total volume:	370	Maximum:	1000
Reagent name	Volume [ul]	Color	Type		
Blood sample	250		Sample		
Lysis Buffer	100		Reagent		
Proteinase K working solution	20		Reagent		
Type a name to add new...					

3. Add the second plate to the tree view:
 - a. Click **New**.
 - b. Select *96 DW plate* as the plate type.
 - c. Name the plate *Wash 1-1* and press **Enter**.
4. Enter the reagent information as shown in the following figure:

Figure 44. Entering reagent information to *Wash 1-1*


Reagent information

Delete		Total volume:	800	Maximum:	1000
Reagent name	Volume [ul]	Color	Type		
Wash buffer 1	800		Reagent		
Type a name to add new...					

5. Copy the *Wash 1_1* plate by clicking **Duplicate**.
6. Rename the plate *Wash 1_2*, and press **Enter**. The reagent information remains the same as in the *Wash 1_1* plate.
7. Add the fourth plate, name it *Wash EtOH*, and enter the reagent information as shown:



Figure 45. Entering reagent information to *Wash EtOH*

Reagent information

Delete		Total volume:	800	Maximum:	1000
Reagent name	Volume [ul]	Color	Type		
80% ethanol	800		Reagent		
Type a name to add new...					



8. Add the fifth plate, name it *Wash 2*, and enter the reagent information as shown:

Figure 46. Entering reagent information

Reagent information			
 Delete	Total volume:	1000	Maximum: 1000
Reagent name	Volume [ul]	Color	Type
Wash Buffer 2	1000		Reagent
Type a name to add new...			

9. Add the sixth plate, name it *Elution*, and enter the parameter values as shown:

Figure 47. Entering reagent information

Reagent information			
 Delete	Total volume:	150	Maximum: 250
Reagent name	Volume [ul]	Color	Type
Elution Buffer	150		Reagent
Type a name to add new...			

10. Add the last plate for picking up and leaving the tip comb, and name it *Tip Plate*.

You do not need to add reagent information to this plate.

Entering General Protocol Data

- Go to the **Protocol** view.
- Optionally enter the *Kit name* and *Protocol description* information.
 - Click the **Tip** icon in the tree view and select *96 DW tip comb* as the tip type.
 - Select *Tip Plate* as the plate for both the *Pick-Up* and *Leave* steps.

The tip comb is picked up from this plate at the beginning of the protocol, and left on this plate at the end of the protocol.

- Click the *Tip* icon in the tree view.

You are now ready to proceed with adding steps to the protocol.

Adding Steps to the Protocol

This example protocol has nine steps. Add them to the protocol in the given order.

Tip When adding *Mix* steps, click **Show Advanced** to view all parameter fields.

Adding the Lysis Step

1. Click the **Mix** button.
2. Set the values as shown:

Figure 48. Entering *General* values

The screenshot shows the 'General' configuration window for a step. It is divided into three sections: 'Beginning of step', 'Mixing/Heating parameters', and 'End of step'. In the 'Beginning of step' section, there are two checkboxes: 'Precollect' and 'Release beads'. In the 'Mixing/Heating parameters' section, there is a 'Mix time [hh:mm:ss]' field set to '00:05:00' and a 'Speed' dropdown menu set to 'Fast'. In the 'End of step' section, there is a 'Collect beads' checkbox. A 'Step icon' dropdown is also visible on the right side.

3. Rename the step *Lysis*.
4. Click **Plate** in the tree view, and select *Sample* as the plate for the step.

Adding the Pause Step

1. Click the **Pause** button.
2. In the *Message* field, you can enter a message to be displayed to the user, for example, information on the reagents to be dispensed on to the plate.
3. Tick the *Dispense* box, so that the correct plate is turned to the loading position.
4. Fill out the fields as shown:

Figure 49. Entering values

The screenshot shows the 'General' configuration window for a step. The 'Message' field contains the text 'Add 25µl Beads and 400 µl Binding Buffer'. The 'Dispense' checkbox is checked. Below this is a table with a 'Delete' button and a 'Total volume' field showing 425. The table has columns for 'Reagent name', 'Volume [ul]', 'Color', and 'Type'. The 'Total volume' field also shows a 'Maximum' of 1000.

Reagent name	Volume [ul]	Color	Type
KingFisher Magnetic Beads	25	Black	Reagent
Binding Buffer	400	Green	Reagent
Type a name to add new...			

5. Select *Sample* as the plate for this step. By reactivating the *Dispense* step, you can view the all the reagents on the sample plate.

Adding the Bind Step

1. Click the **Mix** button.
2. Set the parameters as shown in the following figure:

Figure 50. Entering values

General

Beginning of step

Precollect

Release beads [hh:mm:ss]: 00:00:00 Speed: Bottom mix

Step icon

Mixing/Heating parameters

Mix time [hh:mm:ss]: 00:00:30 Speed: Bottom mix Heating during mixing Preheat

00:04:30 Fast Block temperature [°C]: 50

00:00:00 (none)

Loop count (1...100): 1

End of step

Postmix

Collect beads, count (1...5): 3 Collect time (0...30 s): 1

3. Change the step icon to *Bind*.
4. Right-click the step in the tree view, and rename the step *Bind*.
5. Select *Sample* as the plate.

Adding the First Wash Step

1. Click the **Mix** button.
2. Set the parameters as shown in the following figure:

Figure 51. Entering values

General

Beginning of step

Precollect

Release beads [hh:mm:ss]: 00:00:15 Speed: Bottom mix

Step icon

Mixing/Heating parameters

Mix time [hh:mm:ss]: 00:01:00 Speed: Half mix Heating during mixing Preheat

00:00:00 (none) Block temperature [°C]: 50

00:00:00 (none)

Loop count (1...100): 1

End of step

Postmix

Collect beads, count (1...5): 3 Collect time (0...30 s): 1

3. Change the step icon to *Wash*.
4. Rename the step *Wash 1_1*.
5. Select *Wash 1_1* as the plate for this step.

Adding the Second Wash Step

1. Add the second wash by copying *Wash 1_1*.
2. Paste the copy into the *Protocol Steps* tree.

The step is automatically placed at the end of the protocol sequence.

3. Move the copy upwards so that it is just below *Wash 1_1*.
4. Rename the step *Wash 1_2*.
5. Select *Wash 1_2* as the plate.

Adding the Third Wash Step

1. Click the **Mix** button.
2. Set the parameters as shown:

Figure 52. Entering values

The screenshot shows the 'General' configuration window for a step. It includes the following settings:

- Beginning of step:**
 - Precollect
 - Release beads [hh:mm:ss]: 00:00:15
 - Speed: Bottom mix
 - Step icon: [Icon]
- Mixing/Heating parameters:**
 - Mix time [hh:mm:ss]: 00:01:00
 - Speed: Half mix
 - Heating during mixing
 - Preheat
 - Block temperature [°C]: 50
 - Loop count (1...100): 1
- End of step:**
 - Postmix
 - Collect beads, count (1...5): 3
 - Collect time (0...30 s): 1

3. Change the step icon to *Wash*.
4. Rename the step *Wash Ethanol*.
5. Select *Wash EtOH* as the plate.

Adding the Fourth Wash Step

1. Click the **Mix** button.
2. Set the parameters as shown:

Figure 53. Entering values

General

Beginning of step

Precollect

Release beads

Step icon

Mixing/Heating parameters

Mix time [hh:mm:ss]: 00:00:30 Speed: Slow

End of step

Collect beads

3. Change the step icon to *Wash*.
4. Rename the step *Wash 2*.
5. Select *Wash 2* as the plate.

Adding the Elution Step

1. Click the **Mix** button.
2. Set the parameters as shown in the figure below.

Figure 54. Entering values for the elution step

General

Beginning of step

Precollect

Release beads [hh:mm:ss]: 00:00:00 Speed: Fast

Step icon

Mixing/Heating parameters

Mix time [hh:mm:ss]: 00:05:00 Speed: Slow Heating during mixing Preheat

Block temperature [°C]: 72

00:00:00 (none)

00:00:00 (none)

Loop count (1...100): 1

End of step

Postmix [hh:mm:ss]: 00:05:00 Speed: Fast

Collect beads, count (1...5): 3 Collect time (0...30 s): 30

Note To speed up the heating during mixing, tick **Preheat**.

3. Change the step icon to *Elution*.
4. Rename the step *Elution*.
5. Select *Elution* as the plate for the step.

Saving the Protocol

1. Click **Save**.
2. Select where you want to save the protocol.
3. Name the protocol.
4. Click **OK**.

Before running the protocol, you can enter run-specific information, such as sample identifiers and consumable lot information, in the **Run Information** view.

Plate Change Logic

KingFisher Flex and KingFisher 96/MagMAX Express 96 turntable have eight plate stations, but a protocol for these instruments can contain more than eight plates.

Before executing the protocol, note the following rules:

- The instrument prompts you to load the plate from which it will pick up the tips.
- The plate that is used in the first step is the last one to be loaded onto the turntable at the beginning of protocol execution.
- At the end of the run, the plate of the last step is handed out first.
- If more than eight different plates are used in the protocol, the tip comb is picked up before the other plates are required.

Executing the Protocol

Note Verify the protocol with the instrument simulator before running it with an actual instrument.

1. Switch the instrument on.
2. Make sure the instrument has been added to the PC, and that the instrument is connected to the USB port.

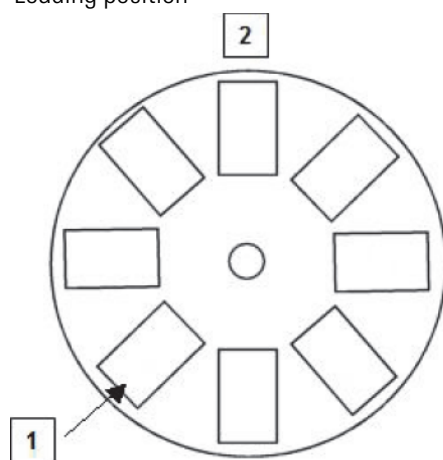
If the instrument has not been added to the PC, follow the instructions in “[Define a New Instrument](#)” on [page 8](#). For further information, refer also to the *Thermo Scientific™ KingFisher™ Flex User Manual*.

3. Click **Connect**.
4. Select the protocol that you wish to run.
5. Click **Start** to launch the protocol directly without transferring it to the instrument memory.
6. Load the plates onto the turntable in the order shown on the instrument display.

The display shows the names of the plates as given in the **Layout** view of the software.

7. Load the *Tip Plate* including the tip comb first into the loading position as requested by the instrument. Load the plate through the sliding door if you are using the see-through lid.

Figure 55. Loading position



Item	Description
1	Loading position
2	Magnet head

8. Press **Start** on the instrument.

The turntable turns, and the display prompts you to load the next plate (*Elution*). Continue until you have loaded all the plates.

9. Press the **Start** button again. The instrument runs the turntable to the correct position and starts executing the protocol.

B Example Protocol: KingFisher Flex

Effective Use of Software

Use BindIt Software faster by using the keyboard and shortcuts.

Right-click Menus

In some cases you can open a context menu by right-clicking the mouse. These menus allow quick access to functions that are relevant to the item you click.

For example, in the **Protocol** view, you can edit, add, and disable steps by right-clicking in the tree view and selecting the right function from the context menu.

The right-click menus are available in the **Layout**, **Protocol**, and **Run Information** views.

Entering Information Without a Mouse

Using BindIt Software requires a mouse, but you can also use your keyboard in certain cases. When you are entering information in the fields (parameters, text fields, check boxes, etc.), you can use:

- The tabulator on your keyboard to move from one field to the next.
- The arrow keys to move sliders and change the available values in the fields.
- The arrow keys to toggle between tabs in a dialog.
- The keyboard shortcuts.

Shortcuts

The list of keyboard shortcuts in BindIt Software is presented below.

Table 10. Keyboard shortcuts

Operation	Keyboard shortcut
Open a protocol.	Ctrl+O
Create a new protocol.	Ctrl+N
Save the protocol.	Ctrl+S
Save the protocol with a new name.	Ctrl+Shift+S
Switch to the Home view.	Alt+H

Table 10. Keyboard shortcuts

Operation	Keyboard shortcut
Switch to the Layout view.	Alt+L
Switch to the Protocol view.	Alt+P
Switch to the Run Information view.	Alt+I
Switch to the Reports view.	Alt+R
Open BindIt Software settings.	Alt+S
Cut the selected string to the Windows clipboard.	Ctrl+X
Copy the selected string to the Windows clipboard.	Ctrl+C
Paste the string from the Windows clipboard.	Ctrl+V
Show online help.	F1