TrueMark™ MSI Assay

For Microsatellite Instability Testing

Catalog Numbers A45295

Pub. No. MAN0018869 **Rev.** A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *TrueMark™ MSI Assay User Guide* (Pub. No. MAN0018868). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Applied Biosystems™ TrueMark™ MSI Assay detects the presence of microsatellite instability in DNA samples through multiplex PCR and fragment analysis. Fragment analysis is performed on the Applied Biosystems™ 3500/3500xL Genetic Analyzer, or the Applied Biosystems™ SeqStudio™ Genetic Analyzer. Data is analyzed with the Applied Biosystems™ TrueMark™ MSI Analysis Software for easy reporting of results.

Set up the PCR reactions

Thaw the TrueMark™ MSI Assay Primer Mix, TrueMark™ MSI Assay Amplification Control, and TrueMark™ MSI Assay No-Template Control on ice. Thoroughly mix the components by vortexing 3–5 seconds, then centrifuge 3–5 seconds before use.

Recommended contents per 10 µL reaction

- TrueMark™ MSI Assay Master Mix —4 µL
- TrueMark™ MSI Assay Primer Mix—1 µL
- DNA—2 to 5 ng

IMPORTANT! Tumor and normal sample pairs should have similar amounts of starting DNA in the PCR reactions to minimize PCR artifacts.

- TrueMark™ MSI Assay No-Template Control— to a final reaction volume of 10 µL.

1. On ice, prepare sufficient PCR reaction mix for the required number of reactions plus 1 additional reaction for overage.

Table 1 Example reaction mix (1ng/ µL DNA)

Component	Volume per reaction	Volume (12 reactions)
TrueMark™ MSI Assay Master Mix	4 µL	48 µL
TrueMark™ MSI Assay Primer Mix	1 µL	12 µL
TrueMark™ MSI Assay No-Template Control	3 µL	36 µL
Total volume	**8 µL**	**96 µL**

2. To the labeled reaction plate, add the following components.
   a. Add 8 µL of PCR reaction mix to each sample, amplification control, or no template control (NTC) well.
   b. Add 2 µL of sample DNA (1 ng/ µL) to the sample wells.
   c. Add 2 µL of TrueMark™ MSI Assay No-Template Control to NTC wells.
   d. Add 1 µL of TrueMark™ MSI Assay Amplification Control and 1 µL of TrueMark™ MSI Assay No-Template Control to the amplification control wells.

   Note: Input DNA quantity and quality affect fragment analysis results.

3. Cover the plate with adhesive film, then centrifuge 3–5 seconds to bring the mixture to the bottom of the tube and eliminate air bubbles.

4. Immediately proceed to "Run the PCR" on page 1.

Run the PCR

1. Program the thermal cycling conditions.

IMPORTANT! If you are using the ProFlex™ 96-well PCR System, select the GeneAmp™ PCR System 9700 simulation mode (**Edit ▸ Manage Steps ▸ Advanced Options ▸ Simulation Mode**).

Step	Temperature	Time	Cycles
Hot start	95°C	11 minutes	1
Denature	94°C	20 seconds	29
Anneal/ Extend	59°C	2 minutes	
Final extension	60°C	25 minutes	1
Hold	4°C	∞	

2. Set the reaction volume to 10 µL, then load the plate into the thermal cycler.

3. Close the heated cover, then start the run.

4. When the run is complete, remove the plate from the thermal cycler.

IMPORTANT! Protect the amplified DNA from light.

Amplified DNA can be stored at 2°C to 8°C for up to 2 weeks, or at –25°C to –15°C for long-term storage.

Prepare your genetic analyzer

The TrueMark™ MSI Assay can be run on the 3500/3500xL Genetic Analyzer or the SeqStudio™ Genetic Analyzer.

1. Download and install the appropriate software.

Instrument	Required software
3500/3500xL Genetic Analyzer	3500 Series Data Collection Software
SeqStudio™ Genetic Analyzer	SeqStudio™ Plate Manager

2. Perform spectral calibration with the DS-36 (Dye Set J6) Matrix Standard Kit.

3. Setup the TrueMark™ MSI Assay run parameters for your system (one time only).
   - 3500/3500xL Genetic Analyzer—Create the instrument protocol, size standards, size standards protocol, and assay.
   - SeqStudio™ Genetic Analyzer—Create the run module, size standards, and plate setup.

   See the *TrueMark™ MSI Assay User Guide* (Pub. No. MAN0018868) for more detailed setup parameters.

Prepare samples for electrophoresis

Prepare the samples for electrophoresis immediately before loading.

1. Prepare the mix of Hi-Di™ Formamide and GeneScan™ 600 LIZ™ Size Standard v2.0 for the required number of reactions plus 1 additional reaction for overage.

Component	Volume per reaction	Volume (12 reactions)
GeneScan™ 600 LIZ™ Size Standard v2.0	1 µL	12 µL
Hi-Di™ Formamide	17 µL	204 µL
Total volume	**18 µL**	**216 µL**

IMPORTANT! The volume of size standard is a suggested amount. Determine the appropriate amount based on your experiments and results.

2. Thoroughly mix the components by vortexing 3–5 seconds, then centrifuge 3–5 seconds before use.

3. Prepare the fragment analysis reactions. To a MicroAmp™ Optical 96-Well Reaction Plate, add the following components.

Component	Volume per reaction	Volume (12 reactions)
Hi-Di™ Formamide and GeneScan™ 600 LIZ™ Size Standard v2.0 mix	18 µL	216 µL
PCR product (see "Run the PCR" on page 1)	2 µL	24 µL
Total volume	**20 µL**	**240 µL**

Note: For blank wells, add 10 µL of Hi-Di™ Formamide.

4. Seal the reaction plate with MicroAmp™ Clear Adhesive Film.

5. Thoroughly mix the components by vortexing 3–5 seconds, then centrifuge 10–20 seconds before use.

6. Denature the DNA fragments:
   a. Incubate the mixture at 95°C for 3 minutes.
   b. Incubate the mixture at 4°C, or on ice, for 2 minutes.

7. Centrifuge the plate for 10–20 seconds to ensure that all sample mixtures are at the bottom of the wells.

8. Remove the MicroAmp™ Clear Adhesive Film, then seal the plate with a septa.

9. Assemble the plate with the retainer and base, then load on the instrument. Reactions can be run on the 3500/3500xL Genetic Analyzer or the SeqStudio™ Genetic Analyzer.

See the instrument user guide for specifics on setting up the run.

Analyze the data with the TrueMark™ MSI Analysis Software

For information on data analysis or troubleshooting with GeneMapper™ Software, see the *TrueMark™ MSI Assay User Guide* (Pub. No. MAN0018868).

Sign in to the TrueMark™ MSI Analysis Software

1. Launch the TrueMark™ MSI Analysis Software.
   - In the Windows™ desktop, click ▸ **Applied Biosystems** ▸ **MSI Client**.
   - On the computer desktop, double-click **(MSI Client)**.

2. Enter the **Username**, then **Password**.

3. Click **Log in**.

Import sample data

1. In the TrueMark™ MSI Analysis Software home screen, click **Import Samples**.
2. Select the FSA files you want to import, or copy, then paste, the filepath to a folder of files in the **File name** field.
3. Click **Import**.
4. (Optional) Enter the Batch Name, Instrument ID, and Operator information.
5. Click **Save**.

Sample naming requirements for TrueMark™ MSI Analysis Software

To be successfully imported into the TrueMark™ MSI Analysis Software, the sample file (FSA) names must follow the correct naming conventions.

In the following examples, "SpecimenID" becomes the main name for identifying the specimen within the software and exports. SpecimenID text cannot contain an underscore (_), because only the text before the first underscore is imported as the specimen ID.

Paired samples convention:

- SpecimenID_T _*.fsa (tumor tissue sample)
- SpecimenID_N _*.fsa (normal adjacent tissue sample from same individual)

Sample file (FSA) names must meet the following conventions.

Sample type	File naming conventions	Guidelines
Specimen	For paired samples, the naming convention is: • <SpecimenID>_T _<*>.fsa—Tumor tissue sample • <SpecimenID>_N _<*>.fsa—Normal tissue sample from the same individual, adjacent to the Tumor tissue sample where: • <SpecimenID> is user-defined, but is identical in the Normal (**N**) and Tumor (**T**) tissue samples • <*> is user-defined For unpaired samples, the naming convention is: <SpecimenID>_<*>.fsa where: <SpecimenID> and <*> are user-defined	• The <SpecimenID> prefix identifies the specimen within the TrueMark™ MSI Analysis Software and exported file names. Ensure that the <SpecimenID> text does not include an underscore (_). • Within a batch, each <SpecimenID> must be unique, unless two files will be analyzed as a Tumor-Normal sample pair. If <SpecimenID> duplicates are detected, the software imports only the last file, in alphanumeric sort order. For example, if the files are named "SpecimenIDBlue_aaa.fsa" and "SpecimenIDBlue_zzz.fsa", the software imports only "SpecimenIDBlue_zzz.fsa". • **IMPORTANT!** The TrueMark™ MSI Analysis Software will not import a <SpecimenID>_N_<*>.fsa file if there is no matching <SpecimenID>_T_<*>.fsa file to import. However, a <SpecimenID>_T_<*>.fsa file will be imported even if there is no <SpecimenID>_N_<*>.fsa file to import. • **IMPORTANT!** If you are running replicate reactions on the same plate, assign the replicates a unique <SpecimenID> before the **_T** or **_N** to ensure that the replicates are processed correctly within the software. For example: – "Spec1.rep1_T" and "Spec1.rep1_N" – "Spec1.rep2_T" and "Spec1.rep2_N"
Negative control	NEG<*>.fsa where: <*> is user-defined **Note:** The TrueMark™ MSI Assay Amplification Control sample file name must begin with "NEG" to be properly analyzed. For example, "NEGAmp.1_A12_daytimestamp.fsa".	File names that begin with "NEG" are analyzed as negative control samples. The TrueMark™ MSI Analysis Software displays a (Warning) Review Flag if it assigns an **Unstable** call to any reportable marker for a negative control sample.
No template control	NTC<*>.fsa where: <*> is user-defined	File names that begin with "NTC" are no template control samples. The TrueMark™ MSI Analysis Software displays a (Warning) Review Flag if it assigns a call other than **No Call** to any reportable marker for a no template control sample.

Table 2 Examples of files that will or will not import

Files selected for import	Import result
20190917.plate1.tst123_T_A01_datetime.fsa	Tumor/Normal pair of files imported as <SpecimenID> "20190917.plate1.tst123"
20190917.plate1.tst123_N_A02_datetime.fsa	
20190924.plate1.sample1.tumor_T_A02.fsa	Unpaired Tumor file imported as <SpecimenID> "20190924.plate1.sample1.tumor"
20190924.plate1.sample1.normal_N_A01.fsa	Unpaired Normal file is not imported
20190924.plate1.sample1.normal_A01.fsa	Unpaired file imported as <SpecimenID> "20190924.plate1.sample1.normal"
specimen1_injection1.fsa	Both files have the same <SpecimenID>, "specimen1". The software imports only the last file, in alphanumeric sort order. In this example, the software imports only "specimen1_injection2.fsa".
specimen1_injection2.fsa	
specimen2_something.fsa, from the 3500/3500xL Genetic Analyzer	Both files imported as <SpecimenIDs> "specimen2" and "specimen3", because each <SpecimenID> is unique. A single batch can contain specimens from different plates and instrument types.
specimen3_something.fsa, from the SeqStudio™ Genetic Analyzer	
specimen4_T_something.fsa, from the 3500/3500xL Genetic Analyzer	Neither file imported, because Tumor/Normal pair of files from different instrument types is not supported.
specimen4_N_something.fsa, from the SeqStudio™ Genetic Analyzer	
HiDi_something.fsa	Files with HiDi prefix in filename are rejected

Note: Files are resized upon import. The failure of one file to meet the sizing quality threshold may prevent any file in the batch from being imported. Open the **Import Manager** for more information on the files reporting errors.

View and interpret the results

Open the **Batch Summary** tab. The **Batch Summary** is populated when data import is complete.

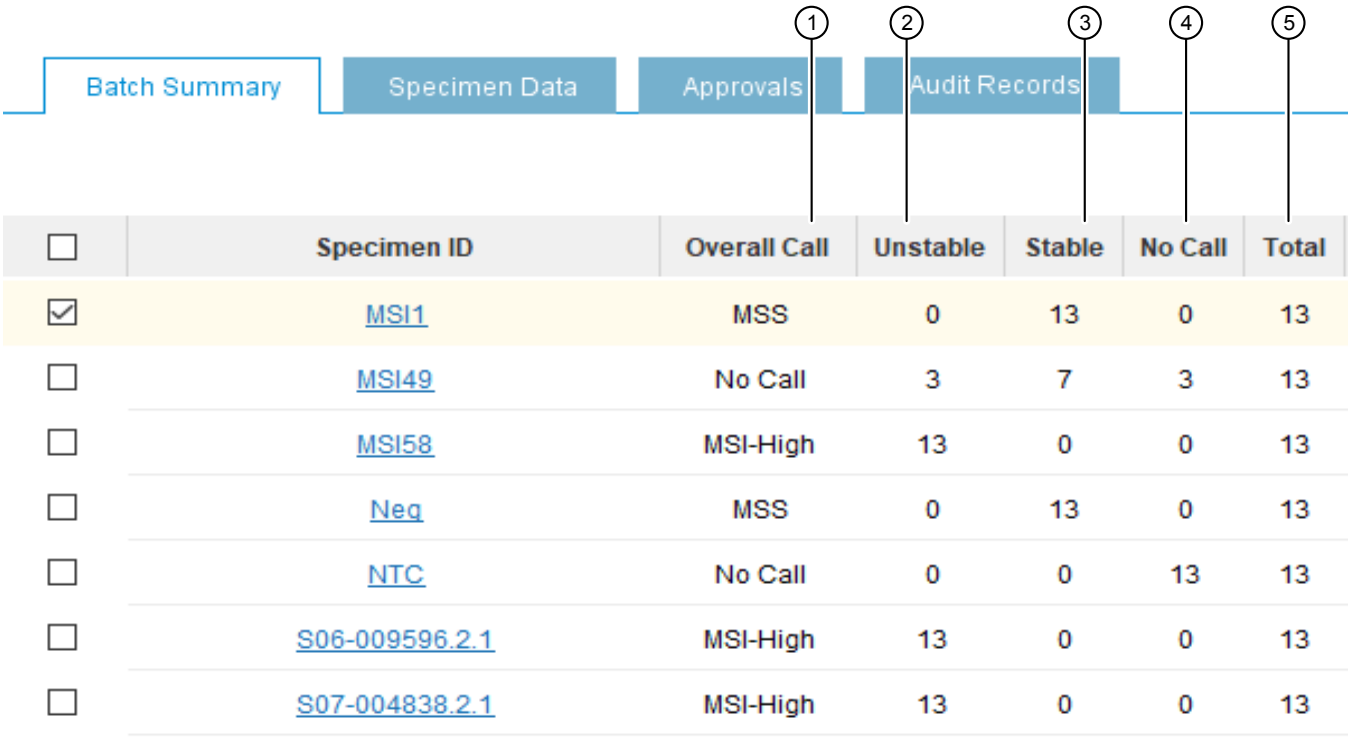
The **Overall Call** for the specimen is based on the percentage of markers reporting instability.

1. View the **Overall Call** for each specimen.
   - **MSS**—Miscrosatellite stable
   - **MSI–Low**—Low levels of microsatellite instability
   - **MSI–High**—High levels of microsatellite instability
   - **No Call**—At least one marker had no call.

     Note: If every marker **No Call** is manually changed to a call of either **Unstable** or **Stable**, then the overall call will be adjusted from **No Call** to **MSS**, **MSI-Low**, or **MSI-High**.

2. View the number of **Unstable**, **Stable**, or **No Call** markers.

Interpret the results



	Specimen ID	Overall Call	Unstable	Stable	No Call	Total
☑	MSI1	MSS	0	13	0	13
☐	MSI49	No Call	3	7	3	13
☐	MSI58	MSI-High	13	0	0	13
☐	Neg	MSS	0	13	0	13
☐	NTC	No Call	0	0	13	13
☐	S06-009596.2.1	MSI-High	13	0	0	13
☐	S07-004838.2.1	MSI-High	13	0	0	13

① **Overall Call** for the specimen
② Number of **Unstable** markers
③ Number of **Stable** markers
④ Number of **No Call** markers
⑤ **Total** number of markers used for the **Overall Call**

See the *TrueMark™ MSI Analysis Software User Guide* (Pub. No. MAN0018874) for more detailed information on viewing individual markers and changing calls.

Generate a report

1. In the **Batch Summary** pane of the batch of interest, click **PDF Report**.
2. Select **Batch Summary** or **Specimen**.

   A message is generated stating **Report generated successfully**. Click **Open folder location** to see where the report was saved, then click **OK**.

Export results

1. In the **Batch Summary** pane of the batch of interest, click **Export Results**.
2. Select the results format to export (**Batch Summary** or **Specimen**).

   Batch Summary results is available in CSV format. Specimen results are available in CSV or VCF formats.

   A message is generated stating **Export(s) generated successfully**. Click **Open folder location** to see where the results were saved, then click **OK**.

Limited product warranty

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For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

The information in this guide is subject to change without notice.

Revision history: Pub. No. MAN0018869

Revision	Date	Description
A.0	13 December 2019	New quick reference guide for the TrueMark™ MSI Assay.