


# Platinum™ Direct PCR Universal Master Mix

**Catalog Numbers** A44647100 (100 reactions), A44647500 (500 reactions), A44647200 (4 × 500 reactions)

**Pub. No.** MAN0018848 **Rev.** A.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

## Product description

Platinum™ Direct PCR Universal Master Mix is a novel master mix ideally suited for amplification directly from sample material without DNA purification. The master mix contains Platinum™ II *Taq* DNA Polymerase that combines inhibitor resistance with Platinum™ hot-start technology for increased specificity. A universal primer annealing feature eliminates the need to optimize annealing temperature for each primer pair.

## Contents and storage

Component	Cat. no. A44647100	Cat. no. A44647500	Cat. no. A44647200	Storage
Platinum™ Direct PCR Universal Master Mix	1 mL	5 mL	4 × 5 mL	-20°C
Lysis Buffer	5 mL	2 × 12.5 mL	8 × 12.5 mL	-20°C or 4°C
Proteinase K	150 µL	750 µL	4 × 750 µL	-20°C
Platinum™ GC Enhancer	40 µL	2 × 1.25 mL	8 × 1.25 mL	-20°C
Water, nuclease free	1.25 mL	5 mL	4 × 5 mL	-20°C, 4°C, or room temperature

## Lysis protocol

The Lysis protocol is an easy method of releasing DNA from a sample. The supernatant from the sample lysate can be used directly for PCR amplification.

### Prepare Lysis Solution

Scale component volumes proportionally according to the amount of Lysis Solution to be prepared (e.g., mix 30 µL of Proteinase K with 1 mL of Lysis Buffer).

1. Add 0.6 µL of Proteinase K to 20 µL of Lysis Buffer.
2. Mix briefly by vortexing, then spin down the solution.  
Lysis Solution stock can be stored at 4°C or -20°C for up to 4 weeks.

### Perform sample lysis

1. Set a heat block to 98°C.
2. Add 20 µL of Lysis Solution to a microcentrifuge tube.  
For larger samples, adjust the volume of Lysis Solution to ensure that the sample will be completely immersed.
3. Add the sample to the tube, then incubate at room temperature for ≥1 minute.

**Note:** The sample can be kept at room temperature for up to 8 hours.

4. Place the sample in the pre-heated heat block and incubate at 98°C for 1 minute.

**Note:** After incubation, the sample can be kept at room temperature for up to 8 hours.

5. Centrifuge the lysate and store at +4°C or -20°C for up to 3 months if not used immediately.

Lysate can be stored with or without the precipitate.

6. Use 1–2 µL of lysate supernatant to “Prepare the PCR Reaction Mix”.

## Prepare the PCR Reaction Mix

1. Thaw the primers, Platinum™ GC Enhancer (optional), and Platinum™ Direct PCR Universal Master Mix.
2. Combine the following components for each sample.

Component	20-µL reaction	Final concentration
Platinum™ Direct PCR Universal Master Mix, 2X	10 µL	1X <sup>[1]</sup>
Forward primer	x µL	0.2 µM
Reverse primer	x µL	0.2 µM
[Optional] Platinum™ GC Enhancer, 5X	4 µL <sup>[2]</sup>	1X
Nuclease-free Water	Fill to 18–19 µL <sup>[3]</sup>	—
Sample supernatant	1–2 µL	—

<sup>[1]</sup> 3.2 mM MgCl<sub>2</sub>.

<sup>[2]</sup> For targets with ≥65 GC content.

<sup>[3]</sup> The final volume after adding supernatant is 20 µL.

3. Mix the PCR Reaction Mix, then centrifuge briefly to bring the contents to the bottom of the tube.
4. Proceed to “Set up and run the PCR instrument”.

## Set up and run the PCR instrument

See the appropriate instrument user guide for detailed instructions to program the thermal-cycling conditions or to run the plate.

1. Program the following thermal cycling conditions into the PCR instrument.

Step	Cycles	Temperature	Time
Activation	1	94°C	2 min
Denaturation	35–40 <sup>[1]</sup>	94°C	15 sec
Annealing		60°C	15 sec
Extension		68°C	20 sec/kb <sup>[2]</sup>
Hold	1	4°C	Hold

<sup>[1]</sup> 40 cycles is recommended for plant and blood samples.

<sup>[2]</sup> For amplicons ≤1 kb, use an extension time of 20 sec. For amplicons > 1 kb use an extension time of 20 sec/kb of the target. Fragments up to 2 kb can be amplified under the same cycling protocol using extension time of the longest fragment.

2. Load the tubes into the PCR instrument, then start the run.

## (Alternative method) Direct PCR protocol

The Direct PCR protocol is used to amplify targets directly from the sample. The protocol is only recommended when primers and template are optimized and well characterized. Fragments of up to 2 kb can be amplified using this method.

### Perform Direct PCR

1. Prepare a tissue sample using a sampling tool or by cutting a very small piece (e.g., a half or whole *Drosophila*) using a sterile scalpel.
  - Tissue samples should be 0.5–1 mm in diameter. Sample size is important and must not exceed 1 mm.
  - Liquid samples (e.g., saliva) should be 1 µL.
2. Combine the following components for each sample.

Component	20-µL reaction	Final concentration
Platinum™ Direct PCR Universal Master Mix, 2X	10 µL	1X <sup>[1]</sup>
Forward primer	x µL	0.2 µM
Reverse primer	x µL	0.2 µM
(Optional) Platinum™ GC Enhancer, 5X	4 µL <sup>[2]</sup>	1X
Nuclease-free Water	Fill to 19–20 µL	—
Sample	1 µL liquid sample <sup>[3]</sup> , or tissue sample <sup>[4]</sup>	—

<sup>[1]</sup> 3.2 mM MgCl<sub>2</sub>.

<sup>[2]</sup> For targets with ≥65 GC content.

<sup>[3]</sup> The final volume after adding supernatant is 20 µL.

<sup>[4]</sup> Ensure that the sample is completely immersed in solution.

3. Perform PCR (see “Set up and run the PCR instrument” for thermal cycler parameters).
4. Add 1 µL of Proteinase K into a 20 µL of PCR reaction before loading the sample on a gel.



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For descriptions of symbols on product labels or product documents, go to [thermofisher.com/symbols-definition](https://thermofisher.com/symbols-definition).

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