invitrogen USER GUIDE

Platinum™ Direct PCR Universal Master Mix

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

Pub. No. MAN0018849 **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**.

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.

Features

- Contains proprietary additives to stabilize primer-template duplexes during the annealing step and allow the use of a standard 60°C annealing temperature for all suitable primer pairs.
- Contains a premixed gel loading dye so that samples can be loaded directly on an agarose gel for analysis after PCR.
- Uses Platinum™ hot-start technology that inhibits DNA polymerase activity at ambient temperatures. This allows reaction set up and storage of pre-assembled PCR mixtures at room temperature. Enzyme activity is restored after the initial denaturation step.
- Includes Platinum™ GC Enhancer that can be added for amplification of GC rich targets (≥65% GC).
- Optimized for amplification from various tissue types and samples.
- Exhibits 5' to 3' polymerase and 5' to 3' exonuclease activity, producing A- tailed PCR fragments.

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

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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.

Guidelines for sample lysis

- Fragments of up to 8 kb can be amplified using this protocol.
- Sample size and amount is not critical.
  - Tissue samples of 0.5–2 mm diameter are sufficient for amplification. Samples up to 1 cm diameter can be used.
  - Liquid samples of 1–2 µL are sufficient for amplification.
- Add samples directly into Lysis Solution and ensure that they are completely immersed.
- Clean sample handling tools with 2% NaClO solution to prevent cross-contamination.

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".

Guidelines for PCR

- Carefully mix and centrifuge all tubes before opening to ensure reagent homogeneity and improve recovery.
- It is unnecessary to perform PCR set up on ice when using Platinum™ Direct PCR Universal Master Mix.
- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.
- Add samples as the last component of the PCR Reaction Mix.
- Annealing temperature with Platinum™ Direct PCR Universal Master Mix is 60°C for most primer pairs designed following standard primer design rules.

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 [1]
Forward primer	*x* µL	0.2 µM
Reverse primer	*x* µL	0.2 µM
(*Optional*) Platinum™ GC Enhancer, 5X	4 µL [2]	1X
Nuclease-free Water	Fill to 18–19 µL [3]	—
Sample supernatant	1–2 µL	—

[1] 3.2 mM $MgCl_2$.
[2] For targets with ≥65 GC content.
[3] 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 [1]	94°C	15 sec
Annealing		60°C	15 sec
Extension		68°C	20 sec/kb [2]
Hold	1	4°C	Hold

[1] 40 cycles is recommended for plant and blood samples.
[2] 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 [1]
Forward primer	*x* µL	0.2 µM
Reverse primer	*x* µL	0.2 µM
(*Optional*) Platinum™ GC Enhancer, 5X	4 µL [2]	1X
Nuclease-free Water	Fill to 19–20 µL	—
Sample	1 µL liquid sample [3], or tissue sample [4]	—

[1] 3.2 mM $MgCl_2$.
[2] For targets with ≥65 GC content.
[3] The final volume after adding supernatant is 20 µL.
[4] 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.

Troubleshooting

Observation	Possible cause	Recommended action
No product or low product yield	Pipetting error.	Repeat PCR reaction set up.
	Inhibition from the sample.	Titrate the amount of sample used for PCR.
		Use the Lysis protocol to prepare the sample.
	Incorrect cycling parameters.	Make sure to use the cycling parameters recommended in the protocol.
	Extension time too short.	The recommended extension time is 20 seconds for amplicons ≤1 kb and 20 s/kb for amplicons >1 kb.
	Not enough PCR cycles.	For difficult amplicons increase number of cycles (up to 40).
	Samples trapped in gel wells.	When performing the Direct PCR protocol, degraded cellular proteins can cause DNA to be trapped in the wells of an agarose gel. To avoid this problem perform the optional Proteinase K treatment step after performing PCR.
	Not enough sample loaded on the gel.	Use at least one third of the PCR reaction volume for gel electrophoresis.
	GC rich sequence.	Add Platinum™ GC Enhancer for sequences with ≥65% GC content.

Observation	Possible cause	Recommended action
No product or low product yield *(continued)*	Primer design.	Perform a control reaction with purified DNA.
		Assess your primer design parameters: • Design 18- to 30-mers with 40–60% GC content. Aim for primer melting temperatures (Tm) between 55°C and 65°C. • Avoid primer pairs with >10°C difference in melting temperature (Tm). • Avoid self-complementary primers and primer pairs complementary at 3' ends. • If possible, design the primers with one or two G or C bases at the 3' end. • Verify primer complementarity to a single template region using programs for sequence alignment. • Use primer design programs available online, such as the OligoPerfect™ Designer primer design tool.
Non-specific PCR products	Contamination with exogenous DNA.	Perform a negative control reaction by adding a no-template control to all PCR reactions.
	Primer design.	Check whether the non-specific product is a primer dimer (when very short fragments are amplified).
		Design new primers following standard primer design recommendations.
	Annealing temperature.	Perform an annealing temperature gradient.
	Cycling protocol.	Reduce the total number of cycles.
		Reduce the extension time.

Guidelines for different sample types

Platinum™ Direct PCR Universal Master Mix was developed to work with cell lines and tissue samples from any organism (human, animal, insect, fish, plant, bacteria etc). Unique master mix composition enables direct amplification from solid and liquid samples (like seeds and saliva). Below are sample specific guidelines that are recommended for optimal performance. Follow protocols above if sample is not mentioned here.

Guidelines for blood samples

Sample type	Guidelines
Blood (Lysis protocol)	• Use 1–20% of blood in total Lysis Solution volume. • Higher blood concentration (up to 40%) can be used, but it may be necessary to adjust the final $MgCl_2$ concentration in the PCR reaction mix from 3.2 mM to 4.7 mM. • Use 1–2 uL of supernatant in 20 µL reaction. • Centrifuge PCR reaction mix at 1000 × *g* for 1–3 min prior to gel analysis.
Blood (Direct PCR protocol)	• Use 1–5% of blood in total reaction volume directly to a 50 µL PCR reaction mix. • Higher blood concentration (up to 10%) can be used, but it may be necessary to adjust the final $MgCl_2$ concentration in the PCR reaction mix from 3.2 mM to 4.7 mM. • Centrifuge PCR reaction mix at 1000 × *g* for 1–3 min prior to gel analysis.

FFPE sample protocol

The DNA in FFPE tissue is often fragmented, limiting the size of PCR products that can be successfully amplified. For best results, select amplicons with a length of <300 bp.

1. Prepare a tube containing 50 µL Lysis Buffer, 1.5 µL of Proteinase K, and 50 µL nuclease-free water.
2. Place a 7–10 µm thick FFPE tissue section into the tube.
3. Crush the tissue with a pipette tip and centrifuge the tube.
4. Ensure the sample is completely immersed in the solution.
5. Incubate at 60°C for 15 minutes.
6. Place the sample in the pre-heated heat block and incubate at 98°C for 5 minutes.
7. Centrifuge the tube at 16,000 × *g* for 2 minutes, then transfer the supernatant into a new tube. Use 1–2 µL of the supernatant as a template in a 20 µL PCR reaction.

Buccal swab sample protocol

1. Prepare a 1.5 ml tube containing 50 µL Lysis Buffer, 1.5 µL Proteinase K, and 250 µL Nuclease-free water.
2. Place the tip of the buccal swab into the tube and swirl the tip 5–10 times in the solution. Remove the swab from the tube and gently pressing the brush against the side of the tube to release absorbed solution.
3. Mix by vortexing and centrifuge the tube.
4. Incubate at room temperature for at least 1 minute.
5. Place the sample in a heat block and incubate at 98°C for 1 minute.
6. Centrifuge the tube and use 2 µL of the sample supernatant as a template for a 20 µL PCR reaction.

Multiplex PCR samples

Follow the lysis protocol to amplify up to 5 fragments of up to 1 kb in length in a single reaction using standard cycling conditions and 0.05 µM of each primer. Calculate extension time based on the longest fragment in the mix. Reaction volume can be increased to 50 µL to add multiple primer pairs. Primer concentration can be increased to 0.5 µM if product yield is low.

It is recommended to verify the performance of each primer pair in a singleplex PCR before starting multiplex reactions. Genomic DNA can be used for primer testing.

Guidelines for genomic DNA control reactions

- Perform a positive control reaction with purified gDNA to ensure that PCR conditions are optimal for your specific primer pair and the target DNA.
- Use 0.5–250 ng of gDNA to perform PCR.

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

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Revision	Date	Description
A.0	9 September 2019	New manual for Platinum Direct PCR Universal Master Mix

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