Certificate of Analysis

PCR Master Mix

Cat.#	Size
M7501	10 reactions
M7502	100 reactions
M7505	1,000 reactions

Description: PCR Master Mix includes Nuclease-Free Water and PCR Master Mix, 2X. PCR Master Mix is a premixed, ready-to-use solution containing *Taq* DNA polymerase, dNTPs, $MgCl_2$ and reaction buffers at optimal concentrations for efficient amplification of DNA templates by PCR.

PCR Master Mix, 2X: 50 units/ml of *Taq* DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400µM dATP, 400µM dGTP, 400µM dCTP, 400µM dTTP, 3mM $MgCl_2$.

Storage Conditions: See the Product Information Label for storage recommendations. Minimize the number of freeze-thaw cycles by storing in working aliquots. Product may be stored at 4°C for up to three months. Mix well prior to use.

Quality Control Assays

Activity Assays

Functional Assay: PCR Master Mix is tested for performance in the polymerase chain reaction (PCR) using PCR Master Mix, 1X, to amplify a 360bp region of the α-1-antitrypsin gene from 100 molecules (0.35ng) of human genomic DNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

***Taq* DNA Polymerase Activity Assay:** *Taq* DNA polymerase activity is confirmed before the enzyme is added to the PCR Master Mix, 2X. The polymerase activity is assayed in 50mM Tris-HCl (pH 9.0); 50mM NaCl; 5mM $MgCl_2$; 200µM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [^{3}H] dTTP); 10µg activated calf thymus DNA and 0.1mg/ml BSA in a final volume of 50µl.

Contaminant Assays

Nuclease Assays: No contaminating endonuclease or exonuclease activity detected.

PCR Satisfaction Guarantee

Promega's PCR Systems, enzymes and reagents are proven in PCR to ensure reliable, high performance results. Your success is important to us. Our products are backed by a worldwide team of Technical Support scientists. Please contact them for applications or technical assistance. If you are not completely satisfied with any Promega PCR product we will send a replacement or refund your account.

That's Our PCR Guarantee!

Product must be within expiration date and have been stored and used in accordance with product literature. See Promega Product Insert for specific tests performed.

Signed by:

R. Wheeler, Quality Assurance

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Promega

Usage Information

1. Description

PCR Master Mix has been optimized for use in routine PCR reactions for amplifying DNA template in the range of 0.2–2kb.

2. Product Components

Product	Size	Cat.#
PCR Master Mix	10 reactions	M7501

Each system contains sufficient reagents to perform ten 50µl reactions. Includes:

- 250µl PCR Master Mix, 2X
- 1.25ml Nuclease-Free Water

Product	Size	Cat.#
PCR Master Mix	100 reactions	M7502

Each system contains sufficient reagents to perform one hundred 50µl reactions. Includes:

- 2 × 1.25ml PCR Master Mix, 2X
- 2 × 1.25ml Nuclease Free Water

Product	Size	Cat.#
PCR Master Mix	1,000 reactions	M7505

Each system contains sufficient reagents to perform one thousand 50µl reactions. Includes:

- 1 × 25ml PCR Master Mix, 2X
- 1 × 25ml Nuclease Free Water

3. Protocol

1. Thaw the PCR Master Mix at room temperature. Vortex the Master Mix and then spin it briefly in a microcentrifuge to collect the material in the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

For a 25µl reaction volume:

Component	Volume	Final Conc.
PCR Master Mix, 2X	12.5µl	1X
upstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
downstream primer, 10µM	0.25–2.5µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	25µl	N.A.

For a 50µl reaction volume:

Component	Volume	Final Conc.
PCR Master Mix, 2X	25µl	1X
upstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
downstream primer, 10µM	0.5–5.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	50µl	N.A.

For a 100µl reaction volume:

Component	Volume	Final Conc.
PCR Master Mix, 2X	50µl	1X
upstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
downstream primer, 10µM	1.0–10.0µl	0.1–1.0µM
DNA template	1–5µl	<250ng
Nuclease-Free Water to	100µl	N.A.

4. General Guidelines for Amplification by PCR

The following guidelines apply to target sequences between 200 and 2,000bp and are optimal for typical thermal cyclers.

A. Denaturation

- Generally, a 2-minute initial denaturation step at 95°C is sufficient.
- Subsequent denaturation steps will be between 30 seconds and 1 minute.

B. Annealing

- Optimize the annealing conditions by performing the reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.
- The annealing step is typically 30 seconds to 1 minute.

C. Extension

- The extension reaction is typically performed at the optimal temperature for *Taq* DNA polymerase, which is 72–74°C.
- Allow approximately 1 minute for every 1kb of DNA to be amplified.
- A final extension of 5 minutes at 72–74°C is recommended.

D. Refrigeration

- If the thermal cycler has a refrigeration or "soak" cycle, the cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.
- This cycle can minimize any polymerase activity that might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

- Generally, 25–30 cycles result in optimal amplification of desired products.
- Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

5. Composition of Buffers and Solutions

PCR Master Mix

50units/ml	*Taq* DNA polymerase [supplied in a proprietary reaction buffer (pH 8.5)]
400µM	each: dATP, dGTP, dCTP, dTTP
3mM	$MgCl_2$

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