

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, MgCl₂ 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 MgCl₂.

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 MgCl₂; 200µM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [³H] 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.

U.S. Pat. No. 6,242,235, Australian Pat. No. 761757, Canadian Pat. No. 2,335,153, Chinese Pat. No. ZL99808861.7, Hong Kong Pat. No. HK 1040262, Japanese Pat. No. 3673175, European Pat. No. 1088060 and other patents pending.

Use of this product for basic PCR is outside of any valid US or European patents assigned to Hoffman La-Roche or Applera. This product can be used for basic PCR in research, commercial or diagnostic applications without any license or royalty fees.

Signed by:

R. Wheeler, Quality Assurance

PCR Master Mix



REF M7502 LOT 0000295188
-30°C -10°C 2019-07-31
Dispensed Lot#: 0000270843
100 reactions

For Laboratory Use

Country of Origin: USA

Promega Corporation
3800 Woods Hollow Road
Madison, WI 53711-5399 USA

Promega



ADM7502 00002951883

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HERE



Promega

Promega Corporation

| | |
|------------------------|-----------------|
| 2800 Woods Hollow Road | |
| Madison, WI 53711-5399 | USA |
| Telephone | 608-274-4330 |
| Toll Free | 800-356-9526 |
| Fax | 608-277-2516 |
| Internet | www.promega.com |

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Part# 9PIM750

Printed in USA. Revised 10/16.

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 | <i>Taq</i> DNA polymerase [supplied in a proprietary reaction buffer (pH 8.5)] |
| 400µM | each: dATP, dGTP, dCTP, dTTP |
| 3mM | MgCl ₂ |