Lumiprobe
Life science solutions

DNA Amplification with ProbeMaster® Lyo GEL PCR Master Mix

ProbeMaster® Lyo GEL is a ready-to-use lyophilized master mix containing all components required for PCR, followed by electrophoretic detection of amplification products. The formulation is optimized to ensure high processivity and amplification specificity. Due to the high density of the mix and the presence of tracking dyes (Bromophenol Blue and Xylene Cyanol), samples do not require the addition of loading buffer prior to gel loading. The presence of two dyes allows precise monitoring of electrophoresis progress.

ProbeMaster® Lyo GEL is suitable for DNA amplification followed by electrophoretic analysis and can be used for routine cloning applications and other workflows requiring downstream use of PCR products (the mix does not contain UDG/dUTP).

Due to the presence of visible-spectrum dyes, ProbeMaster® GEL is not suitable for real-time PCR. For real-time PCR applications, ProbeMaster® Lyo UNI reaction mix is recommended.

Master Mix Composition

- HS Taq DNA polymerase;
- Deoxynucleoside triphosphates;
- PCR buffer (contains Mg^{2+});
- Gel loading dyes;
- Cryoprotectants.

Key Features

- One tube of lyophilized mix after reconstitution with 450 µL of water is sufficient for 100 reactions of 25 µL each.
- The mix is ready to use. Only the DNA template, primers, and water need to be added, significantly reducing setup time. The premixed format minimizes contamination risk.
- Suitable for amplification of DNA fragments up to 3 kb, with GC content ≤70%, not requiring high-fidelity amplification.
- Compatible with genomic, viral, plasmid DNA, and other DNA templates.
- The mix contains a highly processive Hot-Start Taq DNA polymerase activated by 5 min incubation at 95 °C. The HS Taq DNA polymerase is a complex of a monoclonal antibody and an enzyme. Heating during the first PCR cycle inactivates the antibody and activates the enzyme. The Hot-Start technology prevents nonspecific amplification and primer-dimer formation.
- HS Taq DNA polymerase exhibits 5′-3′ polymerase and 5′-3′ exonuclease activities. It also possesses terminal transferase activity, adding a single adenine residue to the 3′ ends of double-stranded DNA, enabling TA cloning of PCR products.
- The composition and density of the mix are optimized for direct loading onto agarose gel after amplification.
- Due to the included dyes, samples can be easily loaded onto agarose gels. The two tracking dyes (Bromophenol Blue and

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Xylene Cyanol) enable precise control of electrophoresis duration.

Applications

Standard PCR, RT-PCR, genotyping, PCR for colony verification, preparation of products for TA cloning, etc.

Equipment Compatibility

Compatible with thermal cyclers of any type.

Protocol

Before starting, add 450 µL of deionized water to the lyophilized mix. Incubate for 1 minute, vortex thoroughly, and briefly centrifuge to collect contents. The reconstituted mix may be stored at 4 °C for up to 30 days or frozen at -20 °C within the stated shelf life. Up to 5 freeze-thaw cycles after reconstitution are permitted.

1. Thaw the master mix if frozen, mix thoroughly, and briefly centrifuge.
2. Prepare the reaction mixture according to the table below, calculated for (N + 0.1N) reactions, where N is the required number of reactions. Mix thoroughly and briefly centrifuge.

- **Calculated per 1 reaction volume of 25 µL***

Component	Volume	Notes
Reaction mix, 5×	5 µL	
Forward primer	0.5–1.5 µL (10 µM)	5–15 pmol per reaction (final concentration 250–750 nM)
Reverse primer	0.5–1.5 µL (10 µM)	
Deionized water	To 25 µL total volume	
DNA template	2–9 µL (cDNA; 50–100 ng genomic DNA; 1–100 pg plasmid DNA)	Add individually to each tube in step 4
Total reaction volume	**25 µL***	When using a different reaction volume, adjust all components proportionally.

* Reaction volume may be adjusted depending on the application; however, volumes below 10 µL are not recommended.

3. Dispense the prepared master mix into PCR tubes without including the DNA template volume.
4. Add 2–9 µL DNA template separately to each tube with a separate pipette tip. After DNA addition, the total reaction volume should be 25 µL. Close the lids of the tubes and spin down the droplets by centrifugation.

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5. Perform DNA amplification using the programs below. Annealing temperature must be optimized individually for each primer pair.

- **If Primer Annealing Temperature ≥60 °C**

Stage	Temperature	Time	Number of cycles
HS Taq activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40
Annealing/Elongation (fluorescence detection step)	60–72 °C	30–60 s	

- **If Primer Annealing Temperature <60 °C**

Stage	Temperature	Time	Number of cycles
HS Taq activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40
Annealing (fluorescence detection step)	55–59 °C	10–15 s	
Elongation	72 °C	15–30 s	

6. Analyze PCR results by agarose gel electrophoresis. No loading buffer is required prior to loading samples into gel wells.

   For detecting amplification products on an agarose gel, use ethidium bromide or a more sensitive, less toxic dye such as dsGreen or dsSafe.

7. If necessary, store amplification products at -20 °C.

Storage conditions

- Storage: 12 months (from delivery date) at 4 °C. Transportation: up to 21 days at temperatures up to 25 °C.
- After reconstitution, store at 4 °C for up to 30 days or at -20 °C within the shelf life. The reconstituted mixture may undergo up to five freeze–thaw cycles.
- Shelf life: 12 months from the delivery date unless otherwise specified in the product passport.

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