DNA Amplification with ProbeMaster® Lyo GEL UDG qPCR Master Mix

ProbeMaster® Lyo GEL UDG is a lyophilized, ready-to-use reaction mixture containing all the necessary components for PCR with subsequent detection by electrophoresis. To reconstitute the mixture into a liquid form, add the specified amount of water. The mixture composition is optimized to obtain ideal results in terms of processivity and amplification specificity. Thanks to the high mixture density and the presence of dyes (Bromophenol Blue and Xylene Cyanol), the sample does not need to be mixed with loading buffer before application to the gel. The presence of two dyes allows for precise control of electrophoresis time.

The ProbeMaster® Lyo GEL UDG reaction mixture is suitable for DNA amplification, with results subsequently detected by electrophoresis. Uracil-DNA glycosylase eliminates contamination from amplicons generated in previous reactions and prevents false-positive results, especially during electrophoresis. Due to the presence of dUTP, the mixture is not suitable for applications where the amplification products need to be used further. For such applications, we recommend using our ProbeMaster®Lyo GEL reaction mixture.

Reaction mixture composition

- HS Taq DNA polymerase;
- Uracil-DNA glycosylase (UDG);
- Deoxynucleoside triphosphate mixture (including dUTP);
- PCR buffer (contains Mg^{2+});
- Dyes for gel loading;
- Cryoprotectants

Key characteristics

- One tube of lyophilized mixture, after dilution in 450 µL of water, is sufficient for 100 reactions of 25 µL each.
- The mixture is completely ready for use. To set up the reaction, only the DNA sample, primers, and water need to be added to the mixture, which significantly saves time. The ready-to-use reaction mixture format reduces the risk of sample contamination.
- Uracil-DNA glycosylase removes amplicon contamination from previous reactions and prevents false-positive results, which is especially important when performing amplicon electrophoresis.
- Suitable for PCR of fragments up to 3000 bp in length, with no more than 70% GC content, and not requiring high-precision amplification.
- Genomic, viral, plasmid DNA, and other templates, as well as cDNA obtained by reverse transcription, can be used.
- Contains a highly processive Hot-Start Taq polymerase, activated at 95 °C for 1 minute. The HS Taq DNA polymerase is a complex of monoclonal antibodies with the enzyme. Heating the sample in the first PCR cycle inactivates the antibodies in the complex and activates the enzyme. The "Hot-Start" technology prevents non-specific amplification and

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primer dimer formation.

- The composition and density of the mixture are optimized for direct application of the sample to an agarose gel after amplification.
- Due to the dyes included in the mixture, samples are easy to load onto an agarose gel. The presence of two dyes (Bromophenol Blue and Xylene Cyanol) allows for precise control of electrophoresis time.

Applications

PCR with detection of amplification products using gel electrophoresis, and PCR after reverse transcription.

Equipment compatibility

Compatible with any thermal cycler.

Protocol

Before starting, add 450 µL of deionized water to the lyophilized mix, wait 1 minute, vortex the contents of the tube, and spin down drops by centrifugation. The reconstituted mix can be stored at 4 °C for 30 days or frozen and stored within the expiration date at -20 °C. The reconstituted mix can be frozen/thawed up to 5 times.

1. Thaw the master mix, vortex thoroughly, and spin down drops by centrifugation.
2. Mix the reaction components according to the table below in the indicated order for (N+0.1N) reactions, where N is the required number of reactions. Vortex the prepared mixture, then spin down the drops by centrifugation.

   Calculation per 1 reaction of 25 µL volume*:

Component	Volume	Note
Master mix, 5×	5 µL	
Forward primer (10 µM solution)	0.5–1.5 µL	5–15 pmol/reaction (final concentration 250–750 nM)
Reverse primer (10 µM solution)	0.5–1.5 µL	
Deionized water	Add to final reaction volume of 25 µL*	
DNA	2–9 µL (cDNA, 50–100 ng genomic DNA, 1–100 pg plasmid DNA	Add separately to each tube (see step 4)
Total reaction volume	**25 µL***	If using a different reaction volume, recalculate component volumes while maintaining the given proportions

* The reaction volume can be changed depending on the specific task, but working with a reaction volume of less than 10 µL is not recommended.

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3. Dispense the prepared mix (excluding the volume of the DNA sample) into PCR tubes.
4. Add DNA samples separately to each tube, and spin down drops by centrifugation.
5. Perform DNA amplification using the programs below (primer annealing temperature is calculated individually for each primer pair).

- **If primer annealing temperature ≥60 °C**

Step	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40
Combined primer annealing and elongation**	60–72 °C	30–60 s	

- **If primer annealing temperature <60 °C**

Step	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40
Primer annealing**	55–59 °C	10–15 s	
Elongation	72 °C	15–30 s	

** If applicable, fluorescence detection should be performed at this stage.

6. Analyze PCR results by gel electrophoresis. NO loading buffer needs to be added to the sample before loading into gel wells.

   For detection of amplification products on an agarose gel, use ethidium bromide or more sensitive and less toxic dyes such as dsGreen *and* dsSafe*.*
7. If necessary, store amplification products at -20 °C.

Storage Conditions

- Storage: 12 months from delivery date at temperatures up to 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 until the expiration date. The reconstituted mix retains its functional properties after 5 freeze-thaw cycles.

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