DNA Amplification with ProbeMaster® Lyo UDG qPCR Master Mix

ProbeMaster® Lyo UDG is a lyophilized reaction mixture containing all the necessary components for PCR. To reconstitute the mixture into a liquid form, simply add the specified amount of water. The composition of the mixture includes Hot-start polymerase and is optimized to achieve optimal amplification processivity and specificity. Uracil-DNA glycosylase eliminates contamination from amplicons generated in prior reactions, preventing false-positive results.

The ProbeMaster® Lyo UDG mixture is suitable for both quantitative PCR and DNA amplification followed by electrophoresis detection. For qPCR reactions, fluorescence detection should use a DNA probe labeled with a fluorophore and quencher (hydrolyzable probes, "molecular beacons", "scorpion" primers) or two probes labeled with fluorophores that form a FRET pair. In addition to DNA probes, the intercalating dye dsGreen can be used for fluorescence detection.

One tube of lyophilized mixture, after dilution in 450 µL of water, is sufficient for 100 reactions of 25 µL each.

Reaction mixture composition

- HS Taq DNA polymerase;
- Uracil-DNA glycosylase (UDG);
- Deoxynucleoside triphosphate mixture (including dUTP);
- PCR buffer (contains Mg^{2+} at a concentration of 3 mM in 1× reaction mixture);
- Cryoprotectants

Applications

Qualitative and quantitative PCR with detection of amplification products both in real-time and using gel electrophoresis; reverse transcription PCR.

For cloning and other applications requiring further work with the PCR product after amplification, we recommend using our ProbeMaster® Lyo UNI product, which does not contain dUTP.

Equipment compatibility

Compatible with all types of thermal cyclers.

Lumiprobe Corporation
115 Airport Dr Suite 160
Westminster, Maryland 21157
USA
Phone: +1 888 973 6353
Fax: +1 888 973 6354
Email: order@lumiprobe.com

Lumiprobe GmbH
Feodor-Lynen-Strasse 23
30625 Hannover
Germany
Phone: +49 511 16596811
Fax: +49 511 16596815
Email: de@lumiprobe.com

Lumiprobe RUS Ltd
Kotsyubinsky street, 4
121351 Moscow
Russian Federation
Phone: +7 800 775 3271
Email: ru@lumiprobe.com

Lumiprobe Limited
Suite 12, 3/F, Great Eagle Centre
23 Harbour Road, Wan Chai
Hong Kong
Mob.: +852-5929-0488 (from HK)
Phone: +86-147-14316277 (from China)
Email: hk@lumiprobe.com

Lumiprobe LTD
2 Tuvim St.
3223562, Haifa
Israel
Phone: +972-(0)4-374-0377
Email: il@lumiprobe.com

Lumiprobe Co., Ltd.
10H-11, Shenmao Commercial Center
No. 59 Xinwen Rd., Meiling Community
Lianhua Street, Futian District
Shenzhen, China
Phone: +86-1471431-6277
Email: cn@lumiprobe.com

Protocol

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

1. Thaw the reaction mixture at room temperature, mix thoroughly, and spin down drops by centrifugation.
2. Mix the reaction components according to the table below in the specified sequence based on (N+0.1N) reactions, where N is the required number of reactions. Vortex the prepared mixture and spin down drops by centrifugation.

   ! To obtain reproducible PCR results, it is recommended to perform reactions in two or more replicates for each DNA sample.

- **Calculation for 1 PCR reaction of 25 µL with real-time detection:***

Component	Volume	Note
5x PCR/qPCR Master Mix with UDG	5 µL	
Forward primer	0.5–1.5 µL of 10 µM solution	5–15 pmol/reaction (final concentration 200–600 nM)
Reverse primer	0.5–1.5 µL of 10 µM solution	5–15 pmol/reaction (final concentration 200–600 nM)
Probe *or*	0.25–0.75 µL of 10 µM solution	2.5–7.5 pmol/reaction (final concentration 100–300 nM)
Intercalating dye	According to manufacturer's recommendation	
Deionized water	Add to final reaction volume of 25 µL*	
DNA	2–9 µL (cDNA, 30–100 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.

- **Calculation for 1 PCR reaction of 25 µL with gel electrophoresis detection:***

Component	Volume	Note
5x PCR/qPCR Master Mix with UDG	5 µL	
Forward primer	0.5–1.5 µL of 10 µM solution	5–15 pmol/reaction (final concentration 200–600 nM)
Reverse primer	0.5–1.5 µL of 10 µM solution	5–15 pmol/reaction (final concentration 200–600 nM)

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Hong Kong
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Email: hk@lumiprobe.com

Lumiprobe LTD
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3223562, Haifa
Israel
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Email: il@lumiprobe.com

Lumiprobe Co., Ltd.
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No. 59 Xinwen Rd., Meiling Community
Lianhua Street, Futian District
Shenzhen, China
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Email: cn@lumiprobe.com

Component	Volume	Note
Deionized water	Add to final reaction volume of 25 µL*	
DNA	2–9 µL (cDNA, 30–100 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 vary depending on the task, but performing the reaction in a volume less than 10 µL is not recommended.

3. Add the prepared mixture (excluding the DNA sample volume) to PCR tubes.
4. Add DNA samples separately to each tube, close the tube/strip caps or seal the plate with film, 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 & elongation (fluorescence detection should occur at this step)	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 (fluorescence detection should occur at this step)	55–59 °C	10–15 s	
Elongation	72 °C	15–30 s	

6. When using an intercalating dye, after amplification, to ensure the absence of nonspecific amplification, it is recommended to perform amplicon melting in the range from 60 to 95 °C.
7. To analyze PCR results by gel electrophoresis: mix samples with gel loading buffer, load them into gel wells, and perform electrophoresis.
8. If necessary, store amplification products at -20 °C.

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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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