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DNA Amplification with ProbeMaster® Lyo ROX qPCR Master Mix

ProbeMaster® Lyo ROX is a lyophilized master mix containing all components required for quantitative PCR using intercalating dyes or hydrolysis probes. The formulation is optimized to deliver reliable results with low threshold cycle values and a high signal-to-background ratio. The ready-to-use format minimizes the risk of sample contamination.

The ProbeMaster® Lyo ROX master mix enables a wide range of applications while reducing preparation time. Due to the presence of the ROX reference dye, the mix is suitable for accurate quantification of DNA template in samples and can be used for quantitative analysis, gene expression studies, genotyping, SNP detection, and other applications.

Master Mix Composition

- HS Taq DNA polymerase
- Deoxynucleoside triphosphate (dNTP) mixture
- PCR buffer (contains Mg^{2+})
- ROX reference dye
- Lyophilization stabilizers

Key features

- One tube of the lyophilized mix, after reconstitution in 450 µL of water, is sufficient for 100 reactions of 25 µL each.
- The mix is fully ready to use. Only the DNA sample, primers, and water need to be added to set up the reaction, which significantly reduces preparation time. The ready-to-use format also lowers the risk of contamination.
- The following templates can be used: genomic DNA, viral DNA, plasmid DNA, cDNA obtained after reverse transcription, and others.
- The mix contains a high-processivity Hot-Start Taq polymerase that is activated by incubation for 5 minutes at 95 °C. The HS Taq DNA polymerase is supplied as a complex of monoclonal antibodies with the enzyme. Heating during the first PCR cycle inactivates the antibodies and activates the enzyme. This 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 has terminal transferase activity, adding an extra adenine residue to the 3′ ends of double-stranded DNA, enabling the use of PCR products for TA cloning.
- The mix includes the ROX reference dye for signal normalization. The ROX concentration is optimized for compatibility with most real-time PCR amplifiers.
- The mix does not contain UDG or dUTP.

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

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Possible applications

- Real-time PCR
- PCR with electrophoretic detection
- PCR with cDNA samples after reverse transcription
- Genotyping
- Colony PCR

Equipment compatibility

Compatible with PCR thermocyclers of any type.

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Protocol

Before starting, add 450 µL of deionized water to the lyophilized mix, wait 1 minute, vortex the tube to mix thoroughly, and briefly centrifuge to collect the contents. The reconstituted mix can be stored at 4 °C for up to 30 days or frozen at -20 °C for the product's shelf life. Up to 5 freeze–thaw cycles are allowed.

1. Mix the tube contents thoroughly and briefly centrifuge.
2. Prepare the reaction mixture according to the table below in the indicated order, calculated for (N + 0.1N) reactions, where N is the required number of reactions. Mix the prepared master mix thoroughly and briefly centrifuge.

   Note: For reproducible PCR results, it is recommended to run each DNA sample in at least two replicates.

- **Reaction setup for one 25 µL reaction (real-time detection)**

Component	Volume	Notes
PCR Master Mix, 5×	5 µL	
Forward primer	0.5–1.0 µL (10 µM)	Final concentration 200–400 nM
Reverse primer	0.5–1.0 µL (10 µM)	
Probe *or*	0.25–0.75 µL (10 µM)	2.5–7.5 pmol per reaction (final concentration 100–300 nM)
Intercalating dye	According to manufacturer's recommendation	
Deionized water	To 25 µL total volume*	Given the volume of the DNA sample to be added in step 4
DNA	2–9 µL (cDNA, 50–100 ng genomic DNA, or 1–100 pg plasmid DNA)	Add separately to each PCR tube in step 4
Total reaction volume:	**25 µL***	If a different reaction volume is used, recalculate the volumes of the reaction components while maintaining the given proportions

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

3. Add the prepared master mix to PCR tubes without the DNA template volume.
4. Add 2–9 µL of DNA or cDNA sample to each tube using a separate pipette tip. After adding DNA, the final reaction volume should be 25 µL. Close the tube caps and briefly centrifuge.

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5. Perform DNA amplification using the programs below (primer annealing temperature should be optimized for each primer pair).

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

Stage	Temperature	Time	Cycles
HS Taq activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40–50
Annealing combined with elongation (fluorescence detection should occur at this step**)	60–72 °C	30–60 s	

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

Stage	Temperature	Time	Cycles
HS Taq activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40–50
Annealing (fluorescence detection should occur at this step**)	55–59 °C	10–15 s	
Elongation	72 °C	15–30 s	

** Select the ROX channel as the passive reference.

6. If an intercalating dye is used, it is recommended that the amplicon be melted between 60 °C and 95 °C after amplification to ensure no nonspecific amplification.
7. For gel electrophoresis analysis, mix PCR products with loading buffer, load them into gel wells, and perform electrophoresis.
8. PCR products may be stored at -20 °C if necessary.

Storage conditions

- 12 months (from delivery) 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 within the product shelf life. The reconstituted mixture may undergo up to five freeze-thaw cycles.
- Shelf life: 12 months from the date of delivery unless otherwise specified in the product certificate.

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