DNA Amplification using the ProbeMaster® Lyo dsGreen 5× PCR/qPCR Reaction Mix

ProbeMaster® Lyo dsGreen is a lyophilized reaction mix containing all the necessary components for performing the Polymerase Chain Reaction (PCR), as well as the intercalating dye dsGreen. The composition of the mix has been optimized to achieve ideal amplification processivity and specificity. To reconstitute the mix into liquid form, simply add the specified volume of water.

The ProbeMaster® Lyo dsGreen reaction mix is suitable for both real-time PCR using the intercalating dye dsGreen and for DNA amplification followed by result detection via electrophoresis. Because the reaction mix lacks UDG/dUTP, it can be used for routine cloning tasks and other applications that require subsequent use of the PCR product.

Reaction Mix Composition

- HS Taq DNA Polymerase;
- Deoxynucleoside triphosphate (dNTP) mix;
- PCR buffer (containing Mg^{2+});
- dsGreen intercalating dye;
- Cryoprotectants

Key Features

- One tube of the lyophilized mix, once reconstituted with 450 µL of water, is sufficient for performing 100 reactions with a volume of 25 µL each.
- The mix is completely ready for use, which reduces the risk of sample contamination and significantly saves time during reaction setup. To set up a reaction, one needs only to add the DNA template, primers, and water to the mix.
- Suitable for PCR amplification of fragments up to 3,000 base pairs (bp) in length, with a GC content not exceeding 70%, and for applications that do not require high-fidelity amplification.
- Genomic, viral, plasmid DNA, and other types of DNA can be used as templates.
- The reaction mix contains Taq polymerase featuring "hot start" technology. The HS Taq DNA polymerase utilized in this product consists of a complex formed between monoclonal antibodies and the enzyme. Heating the sample during the initial PCR cycle inactivates the antibodies within the complex, thereby activating the enzyme. This "hot start" technology effectively prevents nonspecific amplification and primer dimer formation.
- The included HS Taq DNA polymerase exhibits 5′→3′ polymerase, 5′→3′ exonuclease, and adenyltransferase activities, making the resulting PCR products suitable for TA cloning.
- The mixture contains dsGreen, an intercalating dye highly sensitive to the presence of double-stranded DNA, which enables "real-time" PCR (quantitative PCR) without the need for added fluorescent probes.

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- Contains no UDG or dUTP.

Applications

Real-time PCR, PCR with electrophoretic detection, PCR using cDNA templates generated via reverse transcription, genotyping, colony PCR, generation of products for TA cloning, and others.

Equipment Compatibility

Compatible with all types of thermal cyclers.

Protocol

1. Before starting, add 450 µL of deionized water to the lyophilized mixture, wait for 1 minute, mix the contents of the tube, and spin down the drops by centrifugation. Thereafter, the reconstituted mixture can be stored at 4 °C for 30 days or frozen at -20 °C during the shelf life. The mixture may be frozen/thawed up to 5 times after reconstitution.
2. Mix the reaction components according to the table below in the order specified, based on (N + 0.1N) reactions, where N is the required number of reactions. Mix the prepared mixture and spin down the drops by centrifugation.

   ! For reproducible PCR results, it is recommended to set up reactions in at least two replicates for each DNA sample.

- **Calculation per single reaction (25 µL volume*) with real-time detection:**

Component	Volume	Note
PCR reaction mix, 5x	5 µL	
Forward primer	0.5–1.0 µL of 10 µM solution	Final concentration 200–400 nM
Reverse primer	0.5–1.0 µL of 10 µM solution	
Deionized water	Add to a final reaction volume of 25 µL*	Taking into account the volume of DNA sample to be added in step 4
DNA	2–9 µL (cDNA, 50–100 ng genomic DNA, 1–100 pg plasmid DNA)	Added separately to each tube in step 4
Total reaction volume	**25 µL***	If using a different reaction volume, recalculate component volumes while maintaining the specified proportions

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- **Calculation per single PCR reaction (25 µL volume*) with gel electrophoresis detection:**

Component	Volume	Note
PCR reaction mix, 5x	5 µL	
Forward primer	0.5–1.5 µL of 10 µM solution	Final concentration 200–600 nM
Reverse primer	0.5–1.5 µL of 10 µM solution	
Deionized water	Add to a final reaction volume of 25 µL*	
DNA	2–9 µL (cDNA, 50–100 ng genomic DNA, 1–100 pg plasmid DNA)	Added separately to each tube in step 4
Total reaction volume	**25 µL***	If using a different reaction volume, recalculate component volumes while maintaining the specified proportions

*The reaction volume can be adjusted depending on the specific task; however, a reaction volume of less than 10 µL is not recommended for use.

3. Into PCR tubes, add the prepared mixture, **excluding the volume of the DNA sample**.
4. Into each tube, using a separate pipette tip, add 2–9 µL of the DNA/cDNA sample (cDNA, 30–100 ng genomic DNA, 1–100 pg plasmid DNA). After adding the DNA, the total reaction volume should be 25 µL. Close the tube lids and spin down the drops by centrifugation.
5. Perform DNA amplification using the following programs (primer annealing temperature should be calculated individually for each primer pair).

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

Step	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40–50
Combined primer annealing/elongation (fluorescence detection should be performed at this stage**)	60–72 °C	30–60 s	

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

Step	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 min	1
Denaturation	95 °C	10 s	40–50
Primer annealing (fluorescence detection should be performed at this stage**)	55–59 °C	10–15 s	
Elongation	72 °C	15–30 s	

**For fluorescence detection of an intercalating dye, enable acquisition in the FAM channel.

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6. After amplification with real-time result detection, it is recommended to perform an amplicon melt curve analysis in the range from 60 to 95 °C to verify the absence of non-specific amplification.
7. For analysis of PCR results by gel electrophoresis: mix the samples with gel loading buffer, load them into the gel wells, and perform electrophoresis.
8. If necessary, amplification products can be stored at -20 °C.

Storage Conditions

- **Transportation:** At temperatures up to 25 °C for up to 21 days.
- **Storage:** At temperatures not exceeding 4 °C for 12 months within the shelf life.
- **After reconstitution:** Store at 4 °C for up to 30 days, or freeze and store at -20 °C within the shelf life. No more than 5 freeze/thaw cycles of the reconstituted mixture are permitted.
- **Shelf life:** 12 months from the date of delivery, unless otherwise specified in the product certificate.

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