

## DNA Amplification with ProbeMaster® Lyo UNI PCR/qPCR Master Mix

**ProbeMaster® Lyo UNI** is a ready-to-use lyophilized reaction mixture containing all the necessary components for polymerase chain reaction (PCR). To reconstitute the mixture into liquid form, add the specified amount of water.

The ProbeMaster® Lyo UNI mixture is suitable for both real-time PCR and DNA amplification followed by electrophoresis detection. Because UDG/dUTP is not included in the composition, this mixture can be used for routine cloning and other applications that require further use of the PCR product after amplification.

### Reaction mixture composition

- HS Taq DNA polymerase;
- Deoxynucleoside triphosphate mixture;
- PCR buffer (contains  $Mg^{2+}$ );
- Cryoprotectants

### Key characteristics

- One tube of lyophilized mixture, after dilution in 450  $\mu$ L of water, is sufficient for 100 reactions of 25  $\mu$ L each.
- The mixture is ready for use, reducing the risk of sample contamination and significantly reducing setup time for the reaction. For standard PCR (with subsequent analysis by gel electrophoresis), only the DNA sample, primers, and water need to be added to the mixture. For quantitative PCR, an intercalating dye or probe to detect the amplification product, a DNA sample, primers, and water must be added to the mixture.
- For fluorescence detection, use a DNA probe labeled with a fluorophore and a quencher (hydrolyzable probes, “molecular beacons”, “scorpion” type primers), or two probes labeled with fluorophores forming a FRET pair. In addition to DNA probes, the intercalating dye [dsGreen](#) can be used for fluorescence detection.
- Suitable for PCR fragments up to 3000 bp in length, with no more than 70% GC content, and not requiring high-precision amplification.
- Genomic, viral, plasmid DNA, etc., can be used as a template.
- The reaction mixture contains Taq polymerase with “Hot-Start” technology. The HS Taq DNA polymerase used 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 primer dimer formation.
- The HS Taq DNA polymerase included has 5′–3′ polymerase, 5′–3′ exonuclease, and adenylyltransferase activities, allowing the use of PCR products for TA cloning.
- Does not contain UDG and dUTP.

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## Possible applications

Quantitative PCR (qPCR) using intercalating dyes such as dsGreen or hydrolyzable probes, standard PCR (with subsequent analysis by gel electrophoresis), PCR after prior cDNA synthesis, genotyping, colony PCR, product generation for TA cloning, etc.

## Equipment compatibility

Compatible with any thermocycler.

## Protocol

Before starting, add 450 µL of deionized water to the lyophilized mixture, wait 1 minute, mix the contents of the tube, and centrifuge to collect the droplets.

The reconstituted mixture can then be stored at 4 °C for up to 30 days or at -20 °C within the shelf life. No more than 5 freeze/thaw cycles of the mixture are allowed after reconstitution from the lyophilized form.

1. Mix the mixture thoroughly and spin down the droplets by centrifugation.
2. Mix the reaction components according to the table below in the indicated sequence for (N+0.1N) reactions, where N is the required number of reactions. Mix the prepared reaction solution and spin down the droplets by centrifugation.

*! To obtain reproducible PCR results, it is recommended that reactions be run in two or more repeats for each DNA sample.*

• **Calculation for 1 reaction with a volume of 25  $\mu$ L\* with real-time detection:**

Component	Volume	Note
PCR master mix, 5 $\times$	5 $\mu$ L	
Forward primer	0.5–1.0 $\mu$ L of 10 $\mu$ M solution	Final concentration 200–400 nM
Reverse primer	0.5–1.0 $\mu$ L of 10 $\mu$ M solution	
Probe <i>or</i>	0.25–0.75 $\mu$ L of a 10 $\mu$ M solution	Final concentration: 100–300 nM
Intercalating dye	According to the manufacturer's recommendations	
Deionized water	To the total volume of the reaction solution 25 $\mu$ L*	Given the volume of the DNA sample to be added in step 4
DNA	2–9 $\mu$ L (cDNA, 50–100 ng genomic DNA, 1–100 pg plasmid DNA)	Add separately to each PCR tube in step 4
<b>Total reaction volume</b>	<b>25 <math>\mu</math>L*</b>	If a different reaction volume is used, recalculate the volumes of the reaction components while maintaining the given proportions

• **Calculated per 1 PCR reaction of 25  $\mu$ L\* with gel electrophoresis detection:**

Component	Volume	Note
PCR master mix, 5 $\times$	5 $\mu$ L	
Forward primer	0.5–1.5 $\mu$ L of 10 $\mu$ M solution	Final concentration 200–600 nM
Reverse primer	0.5–1.5 $\mu$ L of 10 $\mu$ M solution	
Deionized water	To the total volume of the reaction solution 25 $\mu$ L*	Given the volume of the DNA sample to be added in step 4
DNA	2–9 $\mu$ L (cDNA, 50–100 ng genomic DNA, 1–100 pg plasmid DNA)	Add separately to each PCR tube in step 4
<b>Total reaction volume</b>	<b>25 <math>\mu</math>L*</b>	If a different reaction volume is used, recalculate the volumes of the reaction components while maintaining the given proportions

\*Reaction volume can vary depending on the specific task, but volumes less than 10  $\mu$ L are not recommended.

4. Add the prepared mixture to the PCR tubes without considering the DNA sample's volume.
5. Add 2–9  $\mu\text{L}$  of DNA/cDNA sample (cDNA, 30–100 ng genomic DNA, 1–100 pg plasmid DNA) into each tube with a separate pipette tip. After DNA addition, the total reaction volume should be 25  $\mu\text{L}$ . Close the lids of the tubes and spin down the droplets by centrifugation.
6. Perform DNA amplification using the given programs (primer annealing temperature is calculated individually for each primer pair).

• **If the primer annealing temperature is  $\geq 60$  °C:**

Stage	Temperature	Time	Number of cycles
HS Taq polymerase activation	95 °C	5 min	1
Denaturation	95 °C	10 sec	40–50
Annealing of primers combined with elongation (fluorescence detection should be performed at this stage)	60–72 °C	30–60 sec	

• **If the primer annealing temperature is  $< 60$  °C:**

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

6. If an intercalating dye is used, it is recommended that the amplicon be melted between 60 °C and 95 °C after PCR to ensure no nonspecific amplification.
7. To analyze PCR results by gel electrophoresis, mix the samples with buffer, add them to the gel wells, and perform electrophoresis.
8. If necessary, amplification products can be stored at  $-20$  °C.

## Storage conditions

- Storage: 12 months (from the moment of delivery) 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 stated in the product passport.