DNA Amplification with ProbeMaster® Lyo Eva488 ROX PCR Master Mix

ProbeMaster® Lyo Eva488 ROX is a lyophilized master mix containing all necessary components for polymerase chain reaction (PCR), intercalating dye Eva488, and the reference dye ROX. The composition of the mixture is optimized to obtain ideal results in terms of processivity and specificity of amplification.

The ProbeMaster® Lyo Eva488 ROX master mix is suitable for real-time PCR due to the intercalating dye Eva488. It allows accurate determination of the DNA matrix content in the sample due to the normalizing dye ROX in its composition. The mixture can also be used for DNA amplification with subsequent electrophoresis detection.

Master mix composition

- HS Taq DNA polymerase;
- Deoxynucleoside triphosphates;
- PCR buffer (contains Mg^{2+});
- Eva488 intercalating dye;
- ROX reference dye;
- Cryoprotectants

Key characteristics

- One tube of the lyophilized mixture after reconstitution in 450 µL of water is enough for 100 reactions with a volume of 25 µL.
- The mixture is completely ready for use. Only the DNA sample, primers, and water must be added to the mixture to perform the reaction. It saves considerable time for reaction. The ready-to-use format of the master mix reduces the risk of sample contamination.
- Genomic, viral, plasmid DNA, cDNA after reverse transcription, etc., can be used as a matrix.
- Contains high-processive Hot-Start Taq polymerase with activation for 5 min at 95 °C. The HS Taq DNA polymerase is an enzyme complex with a monoclonal antibody. Heating the sample in the first PCR cycle inactivates the antibodies in the complex and activates the enzyme. The "Hot-Start" technology prevents nonspecific amplification and primer dimer formation.
- HS Taq DNA polymerase has 5′-3′ polymerase and 5′-3′ exonuclease activity; it also has transferase activity: it attaches an additional adenine residue to the 3′ ends of double-stranded DNA, allowing PCR products to be used for TA cloning.
- The master mix contains the intercalating dye Eva488. Eva488 is a dimeric acridine that brightly fluoresces upon binding to double-stranded DNA and does not inhibit the reaction. The fluorescence of Eva488 dye is detected by the FAM channel.
- For signal normalization, the reference dye ROX is included in the master mix. The concentration of ROX has been

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specifically optimized to work on most real-time amplifiers available on the market.

- Does not contain UDG and dUTP.

Possible applications

Real-time PCR, PCR with electrophoresis detection, PCR with cDNA samples after reverse transcription, genotyping, PCR for colony verification.

Equipment compatibility

Compatible with all types of amplifiers.

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Protocol

Before proceeding, add 450 µL of deionized water to the lyophilized master mix, wait 1 minute, voertex the tube contents, and spin down the droplets by centrifugation. The reconstituted mixture can be stored at 4 °C for 30 days or frozen and stored within the shelf life at -20 °C. It is allowed to freeze/thaw the mixture up to 5 times after reconstitution from the lyophilized form.

1. Mix 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.

- **Calculated per 1 reaction volume of 25 µL* with real-time detection:**

Component	Volume	Note
PCR master mix, 5×	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	To the total volume of the reaction solution 25 µL*	Given the volume of the DNA sample to be added in step 4
DNA	2-9 µL (cDNA, 50-100 ng genomic DNA, 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

- **Calculated per 1 PCR reaction of 25 µL* with gel electrophoresis detection:**

Component	Volume	Note
PCR master mix, 5×	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	To the total volume of the reaction solution 25 µL*	Given the volume of the DNA sample to be added in step 4
DNA	2-9 µL (cDNA, 50-100 ng genomic DNA, 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

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

3. Add the prepared mixture to the PCR tubes without considering the DNA sample's volume.
4. Add 2-9 µL 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 µL. Close the lids of the tubes and spin down the droplets by centrifugation.
5. Perform DNA amplification using the given programs (primer annealing temperature is calculated individually for each primer pair).

- **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 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	

** Switch on the FAM channel to detect the fluorescence of intercalating dye. For passive reference, select the ROX channel.

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. 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 delivery date) 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.

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- Shelf life: 12 months from the delivery date unless otherwise stated in the product certificate.

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