Lumiprobe
Life science solutions

DNA Amplification with Lyophilized qPCR Beads

Single Assay qPCR Lyophilized Beads are a pre-formulated and pre-dispensed reaction mixture for carrying out polymerase chain reaction (PCR). Each lyophilized bead contains all the necessary components for PCR and qPCR with a volume of 25 µL.

The composition of the reaction mixture is optimized to obtain ideal results in terms of processivity and specificity of amplification. Lyophilized beads provide excellent reproducibility between reactions by minimizing pipetting steps and reducing the likelihood of pipetting errors and sample contamination. Each batch of the Single Assay qPCR Lyophilized Beads undergoes functional testing to ensure batch-to-batch reproducibility.

The beads are suitable for real-time PCR detection, including quantitative analysis (with fluorescent probes or intercalating dye, e.g., Eva488), as well as for DNA amplification with subsequent detection of results by electrophoresis. The beads are also suitable for routine cloning tasks and other applications that require further handling of the PCR product after amplification.

Single Assay qPCR Lyophilized Beads are compatible with any amplifier and can be used for PCR in amplifiers with classic thermal blocks for PCR tubes and amplifiers with cartridges.

The lyophilized format allows storing the PCR mixture for up to 12 months at temperatures up to 4 °C.

Reaction Mixture Composition:

- hot-start Taq polymerase;
- mixture of deoxynucleoside triphosphates (dNTPs);
- optimized buffer for PCR (contains $Mg2+$ with a concentration of 3 mM in 1× reaction mixture);
- protectors for lyophilization.

Hardware Compatibility:

Lyophilized beads for qPCR are compatible with any type of amplifier and can be used both in amplifiers with classic thermal blocks for PCR tubes and in amplifiers with cartridges.

Possible Applications:

Qualitative and quantitative PCR with real-time detection, amplification followed by gel electrophoresis, PCR after preliminary reverse transcription, genotyping, PCR for checking colonies, obtaining a product for TA-cloning, sequencing, etc.

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Protocol

! The reaction volume may vary depending on the specific application but should always be a multiple of 25 μL.

1. Pre-mix the reaction components, except DNA, in a separate tube, according to the table below, based on (N+0.1N) reactions, where N is the required number of reactions.

- **Calculation for one real-time PCR with a volume of 25 μL:**

Component	Volume	Comments
Upstream primer	0.5–1.5 μL of 10 μM solution	5–15 pmol/reaction (final concentration 200–600 nM)
Downstream primer	0.5–1.5 μL of 10 μM solution	
Probe	0.25–0.75 μL of 10 μM solution	2.5–7.5 pmol/reaction (final concentration 100–300 nM)
or Intercalating dye	According to manufacturer's recommendation	
DNA	2–9 μL	Will be added in step 4 separately to each test tube
Deionized water	Add to a total reaction volume of 24 μL	Taking into account the volume of DNA sample that will be added in step 4.
Total volume of reaction mixture	**24 μL**	When using a different reaction volume, the volumes of the reaction components should be recalculated while maintaining the given proportions

- **Calculation for one PCR with a volume of 25 μL with gel electrophoresis detection:**

Компонент	Объем	Примечание
Upstream primer	0.5–1.5 μL of 10 μM solution	5–15 pmol/reaction (final concentration 200–600 nM)
Downstream primer	0.5–1.5 μL ofnbsp;10 μM solution	
DNA	2–9 μL	Will be added in step 4 separately to each test tube
Deionized water	Add to a total reaction volume of 24 μL	Taking into account the volume of DNA sample that will be added in step 4.
Total volume of reaction mixture	**24 μL**	When using a different reaction volume, the volumes of the reaction components should be recalculated while maintaining the given proportions

2. Wipe clean tweezers with a 70% ethanol solution and dry. Using tweezers, place one bead into each PCR tube.
3. Add the prepared reaction mixture to the beads.

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4. Using a separate pipette tip, add 2-9 μL of DNA/cDNA sample (total 50-100 ng genomic DNA, 1-100 pg plasmid DNA) into each PCR tube. After adding DNA, the total reaction volume should be 25 μL (one bead contributes 1 μL to the reaction volume). Centrifuge the drops.
5. Perform DNA amplification using the given programs (primer annealing temperature is calculated individually for each pair of primers).

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

Stage	Temperature	Time	Number of cycles
Activation of HS Taq polymerase	95 °C	5 minutes	1
Denaturation	95 °C	10 seconds	40–50
Primer annealing combined with elongation	60–72 °C	30–60 seconds	

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

Stage	Temperature	Time	Number of cycles
Activation of HS Taq polymerase	95 °C	5 minutes	1
Denaturation	95 °C	10 seconds	40–50
Primer annealing (Fluorescence detection should be performed at this stage)	55–59 °C	10–15 seconds	
Elongation	72 °C	15–30 seconds	

6. In case of using an intercalating dye, it is recommended to melt the amplicon in the range of 60 to 95 °C after amplification to be sure in the absence of non-specific amplification,
7. To analyze PCR results using gel electrophoresis, mix amplified samples with gel buffer, add them into the gel wells, and perform electrophoresis.
8. If necessary, amplification products can be stored at -20 °C.