DNA Amplification with DryDrops® PCR UDG Lyophilized PCR Beads

Single Assay PCR Lyophilized Beads are a pre-formulated and pre-dispensed reaction mixture for carrying out polymerase chain reaction (PCR). Uracil-DNA glycosylase excludes amplicon contamination from previous reactions and false-positive results. Each lyophilized bead contains all the necessary components for PCR and real-time PCR 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 PCR Lyophilized Beads undergoes functional testing to ensure batch-to-batch reproducibility.

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;
- Uracil-DNA glycosylase (UDG);
- Mixture of deoxynucleoside triphosphates (including dUTP);
- Optimized PCR buffer (contains 3 mM Mg^{2+} in 1× of the reaction mixture)
- Cryoprotectants

Hardware Compatibility:

DryDrops® UDG lyophilized beads are compatible with all types of cyclers and can be used for PCR in both amplifiers with classic thermoblocks for PCR tubes and with cartridges.

Possible Applications:

DryDrops 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 followed by electrophoresis detection.

For cloning tasks and other applications that require further work with the PCR product after amplification, we recommend using our dUTP-free DryDrops® PCR product.

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Protocol

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

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

Calculation per 25 µL reaction with real-time detection:

Component	Volume	Note
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 the 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 the DNA sample that will be added in step 4.
Total volume of the 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 reaction with a volume of 25 µl with gel electrophoresis detection:

Component	Volume	Note
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	
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 the DNA sample that will be added in step 4.
Total volume of the reaction mixture	**24 µL**	When using a different reaction volume, the volumes of the reaction components should be recalculated while maintaining the given proportions

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2. Wipe clean tweezers with a 70% ethanol solution and dry. Use tweezers to place one bead into each PCR tube.

   Important! If you increase the reaction volume, you need to put proportionately more beads in each PCR tube.

3. Add 15–22 µL of the prepared reaction mixture to the beads, assuming that after adding DNA (2–9 µL), the total volume without the bead should be 24 µL.

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 annealing temperature of primers is ≥60°C

Stage	Temperature	Time	Number of cycles
Activation of HS Taq polymerase	95 °C	5 min	1
Denaturation	95 °C	10 s	40–50
Primer annealing combined with elongation (Fluorescence detection should be performed at this stage)	60–72 °C		

If the annealing temperature of primers <60°C

Stage	Temperature	Time	Number of cycles
Activation of HS Taq polymerase	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	

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.

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