

Product Manual Cat. No: #9001

HiDi® DNA Polymerase

Description

HiDi® DNA polymerase is a highly selective DNA polymerase variant, specially evolved for all assays in which **Hi**gh **Di**scrimination is required, for instance in allele-specific PCRs, primer extensions or methylation-specific PCRs.

An aptamer based hot-start formulation of the HiDi® DNA polymerase prevents false amplification. Temperatures above 50–55°C cause the aptamer's secondary structure to melt and will set free the polymerase.

HiDi® DNA polymerase efficiently amplifies from primers that are matched at the 3'-end and discriminates primers that are mismatched.

Applications include SNP-detection by allele-specific amplification (ASA) / allele specific PCR, methylation specific PCR (MSP), HLA genotyping and multiplex PCR.

Kit components

| Component | S pack* | M pack* |
|------------------------------|-------------|-------------|
| HiDi® DNA Polymerase | 50 μL | 200 μL |
| 10x HiDi® Reaction Buffer | 1 x 1.25 mL | 2 x 1.25 mL |

^{*}Other pack sizes, bulk orders and customization are available upon request.

Storage and shipment

Transport with cool packs. The reagents should be stored at -20°C upon arrival. The reagents are stable until the expiration date if stored correctly.

Reaction Master Mix set-up

The recommended master mix set-up for a 50 μ L reaction volume is shown in the table below.

| Reagent | Volume (μL) | Final concentration |
|----------------------------------|--------------------------|-----------------------|
| HiDi® DNA Polymerase (5 U/µL) | 0.5 | 2.5 U/rxn |
| HiDi® buffer (10x) | 5 | 1x |
| ∞Forward primer (10 µM) | 1 | 0.2 μM (0.05–1 μM) |
| ∞Reverse primer (10 µM) | 1 | 0.2 μM (0.05–1 μM) |
| dNTPs (2 mM) | 5 | 200 μΜ |
| Template/Sample extract | x | <1000 ng* DNA |
| Nuclease-free water | Up to 50 μL final volume | |

Keep all components on ice.

Spin down and mix all solutions carefully before use.

∞Primers should ideally have a GC content of 40–60% typically.

*Suggested template concentration should be about 10 ng – 1000 ng (genomic DNA) or 1 pg – 1 ng (plasmid/viral DNA) per reaction.

Instrument and program set-up

| Cycles | Steps | Temperature | Time |
|--------|-------------------------|-------------|-------------------|
| 1 | Initial denaturation | 95°C | 2 min |
| 25–40 | Denaturation | 95°C | 15 sec |
| | Annealing* | 54-72°C | 30 sec |
| | Extension | 72°C | 30 sec /250 bp |

^{*}Typically, the annealing temperature is about 3–5°C below the calculated melting temperature of the primers used.



Product Manual Cat. No: #9001

Technical information and support

HiDi® 10x buffer is optimized for short amplicon length (about 60–200 bp). In case of longer amplicons (>500 bp) the addition of magnesium (+ 0.5–1.5 mM) might be needed.

HiDi® DNA polymerase can be used for real-time cycling, by adding a suitable real-time PCR dye.

HiDi® DNA polymerase is a nuclease deficient DNA polymerase, therefore not suitable for hydrolysis probebased assays. For those assays, HiDi® Taq DNA polymerase (#9201) is recommended.

For technical enquiries or assay development support, please contact us via e-mail at: mdx@medixbiochemica.com.

Additional information and technical resources are available on our website at: info.medixbiochemica.com/resources.