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HiDi® 2x PCR Master Mix

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Store at -20°C.

Contents

HiDi® 2x PCR Master Mix contains all the components necessary for a successful and reliable PCR or primer extension reaction in all standard PCR cyclers, including HiDi® (**Hi**gh **Di**scrimination) DNA polymerase and an optimized buffer including ultrapure dNTPs. Only primers and template need to be added.

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.
HiDi® efficiently discriminates primers, which are not perfectly matched at the 3'-end. 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.

Applications

- SNP-detection by allele-specific amplification (ASA) / Allele-specific PCR
- HLA genotyping
- Multiplex PCR
- Methylation specific PCR (MSP)

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
HiDi® 2x PCR Master Mix	10 µl	1x
Primer forward (10 µM)*	0.4 µl	0.2 µM (0.05-1 µM)
Primer reverse (10 µM)*	0.4 µl	0.2 µM (0.05-1 µM)
Template/Sample extract	x µl	<1000 ng** DNA
Nuclease-free water		up to 20 µl total vol

* Primers should ideally have a GC content of 40-60% typically

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

Typical 3-step PCR protocol

Initial denaturation	95°C	2 min	
Denaturation	95°C	15 sec	25-40 cycles
Annealing*	54–72°C	30 sec	
Extension	72°C	30 sec/250 bp	
Hold	<10°C		

* Typically, the annealing temperature is about 3-5°C below the calculated melting temperature of the primers used.

Quality Control Assays

HiDi® 2x PCR Master Mix is tested for successful ASA performance detecting a genomic SNP (rs72921001) in HeLa genomic DNA. The activity of HiDi® DNA polymerase is monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and a DNA primer. Enzyme concentration is determined by protein-specific staining. Please inquire more information at info@mypols.de for the lot-specific concentration. No contamination is detected in standard test reactions.

Material Safety Data (MSDS)

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC, 1999/45/EC and 1272/2008 (CLP Regulation) any products which do not contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic, do not require a MSDS. However, we recommend the use of gloves, lab coats and eye protection when working with these or any other chemical reagents. myPOLS Biotec takes no liability for damage resulting from handling or contact with this product. This product is not hazardous, not toxic, not IATA-restricted. Product is not from human, animal or plant origin. The source of the product is recombinant protein expression in *E. coli*. The product is for research use only and may be used for *in-vitro* experiments only.

Important notes

- Keep all components on ice.
- Spin down and mix all solutions carefully before use.
- 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.
- The master mix can be used for real-time cycling by adding a suitable real-time dye.
- HiDi® DNA polymerase is nuclease deficient, therefore not suitable for hydrolysis probe-based assays. In this case, HiDi® Taq 2x PCR Master Mix (#4200) is recommended.

References

HiDi® DNA polymerase is based on:
Variants of a Thermus aquaticus DNA Polymerase with Increased Selectivity for Applications in Allele- and Methylation-Specific Amplification. PLoS ONE 2014; 9(5): e96640. M. Drum, R. Kranaster, C. Ewald, R. Blasczyk, and A. Marx.
For more references see www.mypols.de

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