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HiDi® Taq 2x PCR Master Mix

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Store at -20°C.

Contents

HiDi® Taq 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® Taq DNA polymerase and an optimized buffer including ultrapure dNTPs. Only primers and template need to be added.

Description

HiDi® Taq 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® Taq DNA polymerase efficiently amplifies from primers that are matched at the 3'-end and discriminates primers that are mismatched. An aptamer-based hot-start formulation of the HiDi® Taq 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® Taq variant has a 5'-3'-nuclease activity and therefore can be used for hydrolysis probe-based real-time PCRs.

Applications

- SNP-detection by allele-specific amplification (ASA) / Allele-specific PCR
- Genotyping and genomic profiling
- Real-time PCR with fluorescence-based hydrolysis probes
- Real-time multiplex detection PCR

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
HiDi® Taq 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® Taq 2x PCR Master Mix is tested successfully for hydrolysis probe based real-time PCR. The product demonstrates linearity of amplification over a specified serial dilution of human genomic DNA. The activity of HiDi® Taq 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.
- The addition of magnesium (+ 0.5 - 1.5 mM) might be needed in case of longer amplicons >500 bp.
- HiDi® Taq DNA polymerase has a 5'-3'-nuclease activity and therefore can be used for hydrolysis probe-based assays.
- HiDi® Taq DNA polymerase is not suitable for real-time PCRs using a real-time dye such as SYBR Green. In this case, HiDi® 2x PCR Master Mix (#9101) is recommended.

References

HiDi® Taq 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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