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Medix Biochemica

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Product Manual
Cat. No: #9101

HiDi® 2x PCR Master Mix

Description

HiDi® 2x PCR Master Mix is a ready to use reaction mix, containing the HiDi® (High Discrimination) DNA polymerase and an optimized buffer including ultrapure dNTPs. It contains all the components necessary for a successful and reliable PCR or primer extension reaction in all standard PCR cyclers. Only primers and template need to be added.

HiDi® DNA polymerase is a highly selective DNA polymerase variant, specially evolved for all assays in which High Discrimination 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 include SNP-detection by allele-specific amplification (ASA) / allele-specific PCR, HLA genotyping, multiplex PCR, and methylation specific PCR (MSP).

Kit components

Component	S pack*	M pack*
HiDi® 2x PCR Master Mix	1 x 1.25 mL	5 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 25 µL reaction volume is shown in the table below.

Reagent	Volume (µL)	Final concentration
HiDi® 2x PCR Master Mix	12.5	1x
∞Forward primer (10 µM)	0.5	0.2 µM (0.05–1 µM)
∞Reverse primer (10 µM)	0.5	0.2 µM (0.05–1 µM)
Template/Sample extract	X	<1000 ng* DNA
Nuclease-free water	Up to 25 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.

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Technical information and support

HiDi® 2x PCR Master Mix 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 the master mix is not suitable for hydrolysis probe-based assays. For those assays, HiDi® Taq 2x PCR Master Mix (#4200) is recommended.