



www.mypols.de
support@mypols.de
T +49(0)7531 122 965 00



HiDi DNA polymerase

#9001

Store at -20°C.

Contents

HiDi (**H**igh **D**iscrimination) DNA polymerase, 5 U/μl

HiDi reaction buffer, 10x

Description

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. 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

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

Recommendations for PCR/ Reaction Setup

PCR Mix

Component	Volume	Final concentration
Primer forward (10 μM)*	1 μl	0.2 μM (0.05-1 μM)
Primer reverse (10 μM)*	1 μl	0.2 μM (0.05-1 μM)
dNTPs (2 mM)	5 μl	200 μM
HiDi buffer (10x)	5 μl	1x
HiDi DNA polymerase 5 U/μl	0.5 μl	2.5 U/reaction
Template/Sample extract	x μl	
Nuclease-free water		up to 50μl total vol.

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

Typical 3-step PCR protocol

Initial denaturation	95°C	2 min	} 25-40 cycles
Denaturation	95°C	15 sec	
Annealing*	54-72°C	10 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

PCR activity: HiDi DNA polymerase is tested for successful ASA performance detecting a genomic SNP (rs72921001) in HeLa genomic DNA. PCR products are subsequently analysed on a 2.5% agarose gel. A specific product is visualized by ethidium bromide staining at the right amplicon length of 109 bp for the matched primer. In the case of the mismatch primer no product formation is visible after 50 cycles.

DNA polymerase activity: HiDi DNA polymerase activity 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 has been detected in standard test reactions.

Safety

This product does not require a Material Safety Data Sheet because it does neither contain more than 1% of a component classified as dangerous or hazardous nor more than 0.1% of a component classified as carcinogenic. However, we generally 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. Further information can be found in the REGULATION (EC) No 1272/2008 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL.

myPOLS Biotec GmbH, Blarerstraße 56, 78462 Konstanz, Germany, T +49(0)7531 122 965 00

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, but also longer amplicon lengths are possible. The addition of additional Magnesium (+ 0.5 - 1.5 mM) might be needed in case of longer amplicons >500 bp.
- HiDi DNA polymerase can also be used for real-time cycling, when adding a suitable real-time PCR.
- Please note, that HiDi polymerase is a nuclease deficient DNA-polymerase, not suitable for probe-based assays. In this case HiDiTaq DNA polymerase (#9201) 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.

Licences/Patents/Disclaimers

This product is covered by a pending patent application. It is for the purchaser's own internal research use and may not be resold, modified or used for production and commercial purposes of any kind without an agreement with myPOLS Biotec. For information on obtaining additional rights, please contact: info@mypols.de.

The product is for research use only and may be used for in-vitro experiments only.

Product source: recombinant protein expression in E.coli.