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DATA SHEET

Version: 2
Revision date: 08/05/2023

1. Identification

Product name **SNP Taq DNA Polymerase**
(5U/uL) 500U

Cat. No **P0055**

2. Description

SNP PolTaq DNA polymerase (High Discrimination DNA Polymerase) is a highly selective DNA polymerase variant with hotstart properties. It has been selected for assays in which High Discrimination is required, for instance in allele-specific PCRs or methylation-specific PCRs. Whereas many DNA polymerases tolerate mismatched primer-template complexes, SNP PolTaq DNA polymerase efficiently discriminates those and only produces specific amplicons in case of perfectly matched primer pairs. This makes SNP PolTaq DNA polymerase very useful for SNP detections, HLA genotyping or the analysis of single CpG methylation sites.

SNP PolTaq DNA polymerase efficiently discriminates primers, which have a mismatch at the 3'-end. The primer with the mismatch at the 3'-position is the absolute requirement for discrimination of SNPs. If the mutation/mismatch is situated at another place of the primer SNP Pol and SNP PolTaq polymerase will work just as a "normal" Taq polymerase.

3. Composition

Item	Quantity
SNP-Taq DNA Polymerase (5U/µL)	100 µL
PCR Reaction Buffer with $MgCl_2$ (10x)	1 mL

4. Unit definition

One unit of SNP Pol / SNP PolTaq DNA-Polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTPs into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

5. Storage

Store at -20 °C.

6. Applications

- SNP-detection by allele-specific amplification (ASA)
- Allele-specific PCR
- Genotyping and genomic profiling
- Methylation specific PCR (MSP)
- HLA genotyping
- Multiplex PCR

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7. Further information

Product Use Limitation This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handling or from contact with the above product.

Before you start

- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Spin down and mix all solutions carefully before use.
- SNP PolTaq 10X buffer is optimized for short amplicon length (about 60 – 200bp), but also longer amplicons are possible. The addition of additional MgCl2 (0.5 – 1.5mM) might be needed in case of longer amplicons (>500bp).
- SNP PolTaq DNA polymerase can also be used for realtime cycling when adding a suitable realtime PCR fluorescence dye.
- SNP PolTaq DNA polymerase can be used together with probe-based assays.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.

PROTOCOL

1. Thaw 10X buffer, dNTPs or dNTP-mix, primer solutions, and 25mM MgCl2 (if required) at RT or on ice. Keep the solutions on ice after complete thawing. Mix well before use to avoid localized differences in salt concentration.

2. Prepare a master mix according to Table 1. The master mix typically contains all of the components needed for PCR except the template DNA. Prepare a volume of master mix 10% greater than that required for the total number of PCR assays to be performed. A negative control **(without template DNA)** should always be included in every experiment. Keep the master mix on ice.

Recommendations for PCR and qPCR / Reaction Setup (50µL PCR reaction)

Component	Volume	Final concentration
SNP PolTaq DNA Polymerase	0.5 uL	2.5 units/reaction
10X PCR Reaction Buffer with $MgCl_2$	5 uL	1X buffer
dNTP-Mix 10mM	1 uL	0.2 mM
Primer Forward (10uM)*	1 uL	0.2 µM (0.05 – 1.0µM)
Primer Reverse (10uM)*	1 uL	0.2 µM (0.05 – 1.0µM)
Template DNA	x µl	< 10 ng plasmid DNA or <500ng gDNA
Probe**	y uL	0.2 µM (0.05 – 0.3µM)
PCR grade H20	up to 50 µl total reaction volume	-

Keep all components on ice.
Spin down and mix all solutions carefully before use.

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* **Primers should ideally have a GC content of 40-60%. For optimal results we recommend amplicon lengths in the range of 60 to 300bp.**

** **The necessary concentration of probe depends very much on the probe sequence and the kind of probe. Please test for optimum!**

3. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes. Mix gently, for example, by pipetting the master mix up and down. It is recommended that PCR tubes are kept on ice before placing in the thermal cycler.

4. Add template DNA to the individual tubes containing the master mix. For RT-PCR, add an aliquot from the reverse transcriptase reaction. The volume added should not exceed 10% of final PCR volume.

5. Program the thermal cycler according the instructions. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

Step	Time	Temperature	
Initial denaturation:	2 min	95°C	Approximately 3˚C to 5˚C* below lower Tm of primers. approx. 0.5kbp per minute extension rate
3-step cycling:			
• Denaturation	15 sec	95°C	
• Annealing	10 sec	54-72°C	
• Extension	30 sec /250pb	72°C	
Number of Cycles	25-40		
Hold	y uL	< 10°C	