SNPase HotStart Polymerase

Description: SNPase HotStart DNA Polymerase is an modified version of *Taq* DNA polymerase with AntiTaq antibody. A special point mutation in the active site of the enzyme leads to a higher enzymatic specificity and higher precision of incorporation of deoxy- and dideoxynucleotides. Minisequencing SNP genotyping with SNPase HotStart can be carried out by the procedure described in minisequencing protocol (1).

Content

Ref No.	S108210	108210	108250	color
SNPase HotStart Polymerase	**Sample size**	**500 units**	**2500 units**	**blue**
Incomplete * Reaction Buffer (5x)	**1.25 mL**	**1.25 mL**	**2x 1.25 mL**	**red**
$MgCl_2$ 100 mM	**1 mL**	**1 mL**	**2x 1 mL**	**green**
Datasheet	**1**	**1**	**1**	**--**

* Without $MgCl_2$.

Applications: For cycle sequencing with dideoxynucleotides in automated fluorescent DNA sequencing as well as for manual DNA sequencing. SNPase Hotstart is recommended for SNP genotyping by allele-specific PCR (AS-PCR), allele-specific primer extension (AS-PEX) and minisequencing procedures.

Concentration: 20 U/µL

Sensitivity: high

Unit Definition: One unit is defined as the amount of enzyme that incorporates 10nmoles of dNTPs into acid- insoluble form in 30 minutes at 72 °C.

Additionally provided: 1 tube $MgCl_2$ (100 mM)

Recommended $MgCl_2$ concentration: 2.5 mM – 4 mM, recommended for starting is 3.0 – 3.5 mM

Quality Control

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR amplification tests with different templates
- Hotstart efficiency test showing effective blockage by AntiTaq

Storage conditions: -20 °C

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Pipetting scheme

Components	Volume / 25 µL PCR-Reaction	Final concentration
5 x PCR-Buffer (without Mg^{2+})	5 µL	1x
dNTP-Mix (10 mM each)	1 µL	0.2 mM each
$MgCl_2$ (100 mM)	As required	2.5 - 3.5 mM
Upstream Primer	variable	0.1 - 0.5 µM
Downstream Primer	variable	0.1 - 0.5 µM
Template DNA	variable	75 to 125 ng
SNPase Hotstart 5 U/µl	0.25 - 1 µL	1.25 - 5.0 units
Sterile dest. water	Adjust to 25 µL final volume	

Thermocycler protocol

step	time	temperature
initial denaturation	1 - 2 minutes	94 °C
Number of cycles: 30		
denaturation	10 seconds	94 °C
annealing	15 - 30 seconds	59 - 68 °C *
extension	1 minute	68 - 72 °C

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

Notes:
Program the cycler according to the manufacturer's instructions.
Each program should start with an initial denaturation step at 94 °C for 2 to max. 5 min.
Recommended elongation time: 1 min/ 1 kb of target.
For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

(1) Lovmar L, Fredriksson M, Liljedahl U, Sigurdsson S, Syvänen AC. *Quantitative evaluation by minisequencing and microarrays reveals accurate multiplexed SNP genotyping of whole genome amplified DNA.* Nucleic Acids Res. *2003;**31**:e129.*

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