SNPase for SNP-Genotyping

- Hot-Start Polymerase for SNP detection by allele-specific PCR and micro sequencing

Features:
- 10-15 fold lower mutation rate than Taq DNA Polymerase
- high fidelity allele-specific amplification of DNA fragments
- high specificity with lowest background AS-PEX and AS-PCR
- Hot-Start activity for less primer dimers
- only 5'-3' polymerase activity, lack of 5'-exonuclease activity

Applications:
- High specific PCR
- Multiplex PCR
- Real-Time PCR with intercalation dyes
- high fidelity dNTPs and ddNTPs
- *Mini-Sequencing, SNP-genotyping*

Description:
SNPase is Taq DNA Polymerase with unique N-terminal deletion and proprietary amino acids substitutions introduced into the active center of the enzyme. This modification causes dramatic increase of sensitivity of the enzyme to mismatches at 3'-end of the primer. Consequently , non-perfect annealing of the primers does not result in unspecific amplicons formation. This enzyme has only 5'-3' polymerase activity and is recommended for SNP genotyping by allele-specific PCR (AS-PCR), allele-specific primer extension (AS-PEX) and minisequencing procedures.
Minisequencing SNP genotyping with SNPase DNA Polymerase can be carried out by the procedure described in *:
** Reference for minisequencing protocol: 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.*

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.

Concentration:
10-25 u/µl

Reaction Buffer supplied:: 5X Reaction buffer without $MgCl_2$,
1 tube $MgCl_2$ 100 mM

Note:
- optimal $MgCl_2$ concentration: 3.0 -3.5 mM in the 1X reaction mixture
- higher $MgCl_2$ concentrations results in higher yield (up to 4.5 mM)
- lower $MgCl_2$ (2.5 mM) results in higher specificity
- DNA fragments up to 400 bp from Human genomic DNA and 500 bp from Phage-DNA

Usage:

Components	Volume per reaction
5 X reaction buffer without MgCl2	5 µl
$MgCl_2$	2.5 - 4 mM
dNTP-Mix	0.2 mM each
primer mix (5 µM stock)	0,9-1,1 µl (5 pmol)
Template DNA	75-125 ng/25 µl genomic DNA
SNpase	0.2 - 0.5 µl (5-12 units)
Sterile dest. Water (molecular grade)	up to 25 **µl total reaction volume**

General Thermo-Cycler protocol:

Step	Time	Temperature
Initial denaturation	1-2 min	94-95°C
30-35 Cycles: Denaturation Annealing Extension	 20-30 sec 15-30 sec 30-40 sec	 94-95°C 59-68°C 68-72°C per 1kb
Final extension	5 min	72°C

Ordering Information:

Cat.-no	Description	Amount
S500	**SNpase**	500 units
S505	**SNpase**	2500 units