

## SNPase for SNP-Genotyping

### 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'- and 3'- 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 mini-sequencing procedures.

**Minisequencing SNP genotyping** with SNPase DNA Polymerase can be carried out by the procedure described in \*:

\* *Reference for mini-sequencing 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:

20-25 u/μl

### Reaction Buffer supplied:

5X Reaction buffer without MgCl<sub>2</sub>  
MgCl<sub>2</sub> 100 mM

**Note:**

- optimal MgCl<sub>2</sub> concentration: 3.0 -3.5 mM in the 1X reaction mixture
- higher MgCl<sub>2</sub> concentrations results in higher yield (up to 4.5 mM)
- lower MgCl<sub>2</sub> (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                      |
|---------------------------------------|--|
| 5X reaction buffer                    | 5 µl                                     |
| MgCl <sub>2</sub>                     | 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 <b>total reaction volume</b> |

**General Thermo-Cycler protocol:**

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

**Ordering Information:**

| Cat.-no | Description | Amount      |
|---------|-------------|-------------|
| S500    | SNpase      | 500 units   |
| S505    | Snpase      | 5x500 units |

*.. a good decision ..*