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(Revised 10/04/2011)

SNPase Hot Start and 5xReaction Buffer (with 12.5 mM Mg^{2+})

(Catalog #EP052, #EP053, #EP054)

FOR LABORATORY USE ONLY

Description

SNPase Hot Start is a thermostable DNA polymerase designed for a single-nucleotide polymorphism (SNP) detection. This enzyme has only 5'-3' polymerase activity and lacks exonuclease activity. SNPase Hot Start DNA Polymerase provides very high enzymatic specificity and high fidelity incorporation of dNTPs and ddNTPs that is 10-15 times higher than a regular *Taq* DNA Polymerase. The enzyme has a modified polymerase activity that is restored during the initial denaturation step allowing for a hot-start PCR and eliminates the presence of non-specifics products. SNPase Hot Start DNA polymerase is recommended for SNP genotyping by allele-specific PCR (AS-PCR), allele-specific primer extension (AS-PEX) and mini sequencing procedures. The enzyme also can be used for high fidelity amplification of short DNA fragments up to 500 bp from Human genomic DNA and up to 1000 bp from plasmid DNA. SNPase Hot Start DNA Polymerase is supplied at 12 u/µl and comes with an optimized 5xReaction buffer (with 12.5 mM Mg^{2+}) and 100 mM $MgCl_2$. The purified enzyme has no detectable endonuclease or exonuclease activity.

Features

- High sensitivity to mismatches at 3'-end of primers.
- High fidelity of dNTPs and ddNTPs incorporation.
- Lack of 5'-3' and 3'-5'exonuclease activity.
- High specificity, low level of background PCR.
- Fast activation (5-10 sec, 95°C).
- TA cloning.

Applications

- SNP- genotyping, allele specific PCR, allele specific primer extension, mini-sequencing.
- High fidelity PCR (up to 1000 bp).
- Multiplex PCR.
- Real Time PCR with intercalating dyes (SYBR Green, Eva Green).

Limitations

- SNPase can't be used in TaqMan assay due to the lack of 5'- exonuclease activity.
- SNPase can't be used for effective amplification of templates longer than 1000 bp.

SNPase Hot Start concentration - 12 u/µl.

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmols of dNTPs into acid-insoluble material in 30 minutes at 72°C under the assay conditions.

Additional reagents supplied with SNPase Hot Start DNA Polymerase

5xReaction Buffer with 12.5 mM Mg^{2+}
100 mM $MgCl_2$
Note. If precipitation occurs during storage at freezing temperatures, it should be dissolved before usage.

Magnesium Concentration

SNPase Hot Start DNA polymerase provides successful PCR amplification in a range of Mg^{2+} concentration from 2.5 mM to 4 mM in the 1x reaction mixture. Supplied 5xReaction Buffer (with 12.5 mM Mg^{2+}) has low magnesium concentration that favors specific amplification products formation. Increase of Mg^{2+} concentration from 2.5 mM up to 4 mM can provide higher yield of PCR products. Mg^{2+} concentration can be easy optimized for your PCR by supplied 100 mM $MgCl_2$ solution.

Storage and Dilution Buffer

20 mM Tris-HCl (pH 7.5 at 25°C), 100 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 50% Glycerol and 0.1% Tween-20.

Storage Conditions

SNPase Hot Start DNA Polymerase can be stored for 6 months at -20°C.

Basic PCR Protocol

(For amplifications of DNA fragments shorter than 1000 bp)

1. Mix gently SNPase Hot Start DNA Polymerase and spin down content of the tube before adding it to the Reaction Mix. Thaw all reagent solutions completely, mix thoroughly before use and keep all components on ice.
2. Combine the components of the Reaction Mix in the tubes for PCR. Recommended final concentrations of components in each sample for PCR amplifications are: 1xReaction Buffer (with 2.5mM Mg^{2+}); 200µM of each dNTP (provided by the user); 0.2 µM of each primer (provided by the user); template DNA (provided by the user); nuclease-free water (provided by the user) and 6-12 units of SNPase Hot Start DNA Polymerase in a 50µl reaction volume. Mix the content of each PCR sample gently.
3. **Guidelines for Thermal Cycling program.** (Researchers can use their own protocols for specific applications.)

	Predenaturation	94-95 °C	1-3 min
20-40 cycles	Denaturation	94-95 °C	15-30 sec
	Annealing	55-68 °C	15-30 sec
	Elongation	72 °C	30-60 sec
Post-PCR (to finish extension of all templates)	Elongation	72 °C	2 min
Store		4° C	until analysis of samples

Notes

The use of reaction master mixes is strongly recommended to avoid pipetting errors due to addition of small volumes (SNPase Hot Start DNA Polymerase). Combine the appropriate multiples of the components (water, 5xReaction Buffer, dNTPs, primers and SNPase Hot Start DNA Polymerase), make aliquots and start reaction by adding the template.

The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers and could vary from 50°C to 72°C based on the primer Tm.

Extension of the primer by SNPase Hot Start DNA Polymerase requires approximately 1 minute per 1000bp of the template to be amplified.

WARNINGS

We recommend the use of lab coats, gloves and eye protection when working with these reagents. MGQuest assumes no liability for any damage resulting from handling or from contact with the above products.

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