

## **SUBTHERM DNA POLYMERASE**

### **FS-T-022: 250 UNITS**

#### **DESCRIPTION:**

SubTherm DNA Polymerase is a thermostable 94 kDa DNA Polymerase purified from E.coli PVG-AI recombinant strain expressing *Thermus aquaticus* polymerase gene.

The enzyme catalyzes polymerisation of nucleotides into duplex DNA in the 5'-3' direction in presence of Mg<sup>++</sup> ions. The enzyme possesses also a 5'-3' exonuclease activity. Amplification of target **DNA fragments from 100 b.p. to 5000 b.p.** can be achieved with this enzyme.

#### **CONCENTRATION:**

5 units/ul

#### **UNIT DEFINITION:**

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

Unit Assay Conditions: 1 x AS buffer, 200 µM dNTPs including [<sup>3</sup>H]-dTTP and 250 µg/ml activated calf thymus DNA

#### **STORAGE AND DILUTION BUFFER:**

20mM Tris-HCL (pH 7.6);100mM KCl;0.1mM EDTA; 1mM DTT; Triton X-100; 50% glycerol

#### **STORAGE TEMPERATURE:**

Store SubTherm DNA Polymerase below 0° C, preferably at -20° C, in a constant temperature freezer.

#### **EXPIRY DATE:**

1 year upon receipt.

#### **10X REACTION BUFFER:**

**NH4-buffer:** 166mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>;670mM Tris-HCL (pH 8.8 at 25°C);0.1% Tween-20.

500 µl 10X Reaction Buffer

10X Reaction Buffer (contains 10 mM MgCl<sub>2</sub>; included)

Cat. No. **FS-B-006**

10X Reaction Buffer (without MgCl<sub>2</sub>; plus 50 mM MgCl<sub>2</sub> separately)

Cat. No. **FS-B-007**

### Protocol for routine Taq PCR reaction.

All components should be mixed and spun down prior to pipetting. These recommendations serve as a starting point; in order to maximize amplification the reaction conditions may require optimization.

Component	Volume (µl)	Final Concentration
Taq Reaction Buffer (10x)	5 µl	1x
Deoxynucleotide Solution Mix (10x)	1 µl	200 µM
Upstream Primer (10 µM stock)	0,5-2,5 µl	0,1-,05 µM
Downstream Primer (10 µM stock)	0,5-2,5 µl	0,1-,05 µM
DNA Template	determined by user	0,1-1 ng/ml plasmid DNA 1-10 µg/ml genomic DNA
Taq DNA Polymerase	0,2 µl	0,02 units/µl
Nuclease free water	Bring reaction to a final volume of 50 µl	

Gently mix the reaction and spin down in microcentrifuge.

If the thermocycler does not have a heated cover, add one drop of mineral oil to the reaction tube to prevent evaporation.

### Cycling conditions for a routine PCR reaction:

Initial Denaturation	95°C	2 - 5 minutes
20 – 30 Cycles	95°C	15 – 30 seconds
	55 - 65°C	15 – 30 seconds
	72°C	1 minute per 1000 base pairs
Final Extension	72°C	5 minutes
Storage	4°C	∞