SuperTaq

Product:	Item Number:	Conc:
SuperTaq DNA Polymerase	Super Taq	5units/µl

Product Information: SuperTaq is relatively more thermostable DNA Polymerase isolated from a strain of Thermus aquaticus. It has a half life of 3 hours at *95* **°C, it** is very stable. It has high fidelity with an error frequency 10 / 10^6 (or 0.01 / 10^3) during DNA synthesis. SuperTaq is designed for use in primer extension reaction. DNA sequencing at high temperature may decrease the second structure of some DNA templates and permit polymerization through base-paired region. DNA sequencing with Taq DNA Polyinerase produces uniform bands intensities and low background.

Quality& Performance Parameters: SuperTaq is highly purified free of contaminating endonucleases, exonucleases and nicking activity. For endonuclease assay, 1 µg of Lambda *I* Hind III DNA is incubated with 20 units of the enzyme in assay buffer at *75°* C for 16 hrs and no visible contaminating activity is observed; For exonucleases assay, 1 µg of pBR322 plasmid DNA is incubated with 10 units of enzyme for 16 hrs at 75° C in assay buffer and no detectable exonuclease is observed. The purity of the enzyme is also evaluated by adding 10 units of SuperTaq in 100 µl of a reaction mixture for making first strand cDNA at beginning and no impaired effect on the first strand is observed.

Unit : One unit incorporates 10 nmole of dNTP into acid-insoluble material in 30 mm. at 74° C.

Concentration in Storage Buffer: 5 units / p1 in 100mM KC1, 20 mM Tris HC1 (pH 8.0, 22°C), 0.lmM EDTA. 0.5mM PMSF, 1mM DTT, 50% glycerol.

10 X Taq Reaction Buffer: (New!) 100 mM KC1, 100mM (NH4)2SO4, 200 mM Tris HC1 (pH 8.75) at 22° C, 1% Triton X-100 and 1mg/ml BSA. Buffer is optimized for use with 200 µM dNTPs.

Magnesium Sulfate: (New!) 20mM $MgSO_4$. The final $MgSO_4$ may be variable according to requirements. Normally 2mM $MgSO_4$ is recommended.

General Reaction mixture for PCR: Taq (5u/ul): 0.5ul, l0xRxn Buffer: l0ul, $MgSO_4$ (20mM): l0ul, dNTP mixture *(2.5mM* each): 8u1, Primer 1: 0.2-1.0um, Primer 2 : 0.2-1 .0uM, Template : 10pg-lug, Sterilized ddH_2O up to l00ul

Primer Extension Characteristics: Taq has the independent terminal transferase activity which results in the addition of a single nucleotide **(** adenosine **)** at 3' end of the extension product. TA cloning vector is recommended if the extension product is needed to be cloned.

Reaction Mixture -Set Up

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Add components, in the following order, into a thin-walled PCR tube. Keep all components on ice. The following control PCR reactions should be performed in parallel with your experiments to ensure that the Super Taq is working properly.

Reagent	Final Conc.	Quantity	Reagent	+ve Control	-ve Control
Water (PCR--Grade)	---	Variable	Water (PCR--Grade)	32.8µl	33. 8µl
10x Taq reaction buffer	1x	5µl	10x Taq reaction buffer	5µl	5µl
$MgSO_4$(20mM)	2-4mM	Variable	$MgSO_4$(20mM)	5µl	5µ1
2.5mM dNTP mixture	200µM of each	4µ1	2.5mM dNTP mixture	4µl	4µl
Primer I, forward	0.l-1µM	Variable	Primer I(l0µM), forward	lµl	1µl
Primer II, reverse	0.1-1 µM	Variable	Primer II(l0µM), reverse	1 µ1	1 µ1
Super Taq	1-1.5u/50µl	Variable	Super Taq(5u/µl)	0.2µ1	0.2µl
Template DNA	See note 1	Variable	Control DNA Template	1 µ1	---
Total Volume	---	50µl	**Total Volume**	50µl	50µl

Storage: -20°C but stable at room temp for several hours

3. Gently vortex the sample and briefly centrifuge to collect all drops from walls of the tube.
4. Overlay the sample with one-half of the total reaction volume of mineral oil or add an appropriate amount of wax. This step may be omitted if the thenno cycler is equipped with a heated lid.
5. Place samples in a thermo cycler and start PCR.

Note for the Components of the Reaction Mixture:

1. ***Template DNA:*** Usually the amount of template DNA is in the range of 0.01-lug plasmid or phage DNA and 0.1-1µg for genomic DNA, for a total reaction mixture of 50µl.
2. ***Primers:*** The PCR primers are usually 15-30 nucleotides in length, longer primers provide higher specificity. The GC content of primer should be 40-60%. The primer should not be self-complementary or complementary to any other primer in the reaction mixture, and the melting temperature of flanking primers should not differ by more than 5°C. If the primer is shorter than 25 nucleotides, the approx. melting temperature^{TM} is calculated using the formula as: **Tm=4(G+C) + 2(A+T).**
3. ***$MgSO_4$ concentration:*** Since Mg^{2+} ions form complex with dNTPs, primers and DNA templates, the optimal concentration of $MgSO_4$ has to be selected for each experiment. In our experiments, at a final dNTP concentration of 200µM. 2mM $MgSO_4$ concentration is suitable in most case.
4. ***dNTPs:*** The final concentration of each dNTP in the reaction mixture is usually 200µM.
5. ***Super Taq:*** Usually 1-1.5u of Super Taq are used in the 50µl of reaction mix. Higher SuperTaq concentrations may cause synthesis of nonspecific products. However, if inhibitors are present in the reaction mix (e.g., if the template DNA used is not highly purified), higher amounts of Super Taq(2-3u)may be necessary to obtain a better yield of amplification products.
6. ***Cycling conditions:*** Usually denaturation for 0.5-2min at 94-95°C is sufficient; the optimal annealing temperature is *5°C* lower than the melting temperature of primer-template DNA duplex; Usually the extending step is performed at *70-75°C*. Recommended extending time is 1min for the synthesis of PCR fragments up to 2kb. When larger DNA fragments are amplified, the extending time is usually increased by 1min for each 1kb.
7. ***Number of cycles:*** The number of PCR cycles depends on the amount of template DNA in the reaction mix and on the expected yield of the PCR product. For less than 10 copies of template DNA, 40 cycles should be performed. if the initial quantity of template DNA is higher, *25-35* cycles are usually sufficient.
8. ***Final extending step:*** After the last cycle, the samples are usually incubated at 72°C for 5-15min to fill-in the protruding ends of newly synthesized PCR products. Also, during this step, the terminal transferase activity of SuperTaq adds extra A nucleotides to the 3'-ends of PCR products.

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