SinaClon
شرکت سیناکلون BioScience

SinaClon Taq DNA Polymerase
(Recombinant)

Products:

SmarTaq DNA Polymerase

Cat. No. DP1611 : 100 units
DP1612 : 500 units
DP1613 : 2500 units
DP1614 : 5000 units

Taq DNA Polymerase

Cat. No. DP1601 : 100 units
DP1602 : 500 units
DP1603 : 2500 units
DP1604 : 5000 units

Concentration: 5unit/µl
Store: -20° C
Shipment: Dry or Wet ice

Description: Taq DNA Polymerase was originally isolated from the thermophilic eubacterium thermus aquaticus BM, a strain lacking Taq restriction endonuclease.The enzyme was cloned in E.coli and is isolated to be free of unspecific endo or exonucleases. The enzyme consists of a single polypeptide chain with a molecular weight of approx 95KD. It is a highly processive 5'-3' DNA polymerase, that lacks 3'-5' exonuclease activity. The enzyme exhibits highest activity at a pH of around 9 (adjusted at 20°C) and temperatures around 75°C. Taq DNA Polymerase activity is stable against prolonged incubations at elevated temperatures (95°C) and can therefore be used to amplify DNA-fragments by the polymerase chain reaction (PCR). Taq DNA Polymerase also accepts modified deoxyribonucleoside triphosphates as substrates and can be used to lable DNA fragments either with radionucleotides, digoxigenin or biotin.

The high processivity, absence of exonuclease activity and temperature optima of Taq DNA Polymerase enable the use of this enzyme in DNA sequencing especially where the resolution of secondary structures plays a major role.

Components:

-50mM Magnesium Chloride and AMS (Ammonium Sulfate)10X PCR Buffer Supplied with **SmarTaq DNA Polymerase.**
-50mM Magnesium Chloride and 10X PCR Buffer supplied with **Taq DNA Polymerase.**
The PCR Buffers are supplied at a 10X Concentration.
10X PCR Buffer Contains 500mM KCl and Tris-HCl (pH 8.4), AMS buffer Contains 200mM Ammunium sulfate, Tris-HCl (pH 8.8).
50mM Magnesium Chloride is supplied in separate tube.

Unit definition:

One unit incorporates 10nmol of deoxyribonucleotide acid-precipitable material in 30 minutes at 74°C.

Application:

PCR amplification **(of DNA fragments as long as 5000bp guarantied by SmarTaq DNA Polymerase)**
DNA labeling
DNA sequencing
PCR for cloning

Storage Buffer:

20mM Tris-HCl (pH 7.9), 0.1mM EDTA, 5mM 2ME, added stabilizers, and 50% glycerin.

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Quality Control:

Absence of endonucleases:

1µl lambda DNA is incubated with 10 units of Taq DNA Polymerase in 50µl test buffer containing 1.5mM $MgCl_2$ for 16 hours at 65°C. No detectable degradation of lambda DNA observed.

1µl Eco/Hind-fragment of lambda DNA is incubated with 10 units Taq DNA Polymerase in 50µl test buffer containing 1.5mM $MgCl_2$ for 16 hours at 65°C. The amount of enzyme showing no alteration of the banding pattern.

Absence of nicking activity:

1µl supercoiled pBR 322 DNA is incubated with Taq DNA Polymerase in 50µl test buffer containing 1.5mM $MgCl_2$ for 4 hours at 65°C.The amount of enzyme showing no relaxation of supercoiled DNA.

Absence of priming activity:

100ng of template DNA is incubated without primers with 10 units Taq DNA Polymerase in 100µl test buffer containing $MgCl_2$ and dNTP under PCR conditions. As analyzed agarose gel electrophoresis, no DNA synthesis occurs.

Heat Stability:

10 units of Enzyme incubated 30min at 95°C and then used in PCR amplification reactions under PCR conditions.As analyzed by agarose gel electrophoresis, DNA synthesis occurs.

Functional assay:

SinaClon Taq DNA Polymerase was tested for amplifications of 977 and 788bp multiplex PCR from human genomic DNA, DNA viruses and amplification of cDNA (RNA viruses).

Basic PCR protocol:

The following basic serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times, temperatures, concentration of Taq DNA Polymerase, primer, MgCl2 and template DNA vary and need to be evaluated by the user).

Add the following components to a sterile 0.5ml microcentrifuge tube sitting on ice:

Components	Volume	Final Concentration
10X PCR buffer(AMS)	10µl	1X
dNTP mix (10mM)	2µl	0.2mM each
$MgCl_2$ (50mM)	3µl	1.5mM
primers (10µM each)	5µl	0.5µM each
Template DNA	1µg	------
Taq DNA Polymerase	0.5µl	2.5unit/100µl reaction
Autoclaved distilled water up to 100µl		

PCR may be perform in 25-35 cycles as follows:

Denaturation	93°C	45 seconds
Annealing	55°C	30 seconds
Extension	72°C	90 seconds

Optimal reaction conditions vary and need to be evaluated by the user.

Mix and centrifuge buffers and Enzyme before opening.

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