Product information

Taq DNA Polymerase High Purity (New Buffer!)

Catalog #: HTD0078
Concentration: 5 U/ul; 1ul contains 5 units Taq DNA Polymerase high purity.
Storage: -20°C
Package:

Taq DNA Polymerase	200U	500U	1000U
NEW 10 X Rxn. Buffer	1.8ml	2x1.8ml	3x1.8ml
NEW 20mM MgSO4	1.8ml	2x1.8ml	3x1.8ml

Product Description:

Taq DNA Polymerase is a thermostable DNA Polymerase isolated from a strain of Thermus sp. Taq has a half life of 3 hours at 95°C, it is therefore more stable than many other DNA Polymerases. Taq has high fidelity with an error frequency 10/10^6 (or 0.01/10^3) during DNA synthesis. Taq 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 Polymerase produces uniform bands intensities and low background.

Performance & Quality Testing:

Taq DNA Polymerase is highly purified free of contaminating endonucleases, exonucleases and nicking activity. For endonuclease assay, 1 ug of Lambda /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, 1ug 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 Taq DNA Polymerase in 100ul of a reaction mixture for making first strand cDNA at beginning and no impaired effect on the first strand is observed.

Unit Definition:

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

Concentration in Storage Buffer:

5 units/ul in 100mM KCl, 20mM Tris HCl (pH 8.0, 22°C), 0.1mM EDTA, 0.5mM PMSF, 1mM DTT, 50% glycerol.

NEW 10 X Taq Reaction Buffer:

100mM KCl, 100mM (NH4)2SO4, 200mM Tris HCl (pH 8.75) at 22°C, 1% Triton X-100 and 1mg/ml BSA. Buffer is optimized for use with 200uM dNTPs.

NEW Magnesium Sulfate:

20mM MgSO4. The final magnesium sulfate may be variable according to individual requirements. In general, **2mM MgSO4 is recommended**.

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 run in parallel to ensure that the Taq DNA polymerase is working properly.

Reagent	Final Concentration	Quantity	Reagent	Positive Control	Negative Control
Water (PCR--Grade)	---	variable	Water (PCR--Grade)	32.8ul	33.8ul
10x Taq reaction buffer	1x	5ul	10x Taq reaction buffer	5ul	5ul
MgSO4(20mM)	2-4mM	variable	MgSO4(20mM)	5ul	5ul
2.5mM dNTP mixture	200uM of each	4ul	2.5mM dNTP mixture	4ul	4ul
Primer I, forward	0.1-1uM	variable	Primer I(10µM), forward	1ul	1ul
Primer II, reverse	0.1-1uM	variable	Primer II(10µM), reverse	1ul	1ul
Taq DNA polymerase	1-1.5U/50ul	variable	Taq DNA polymerase(5u/µl)	0.2ul	0.2ul
Template DNA	See note 1	variable	Control DNA Template	1ul	---
Total Volume	---	50ul	Total Volume	50ul	50ul

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 thermo cycler is equipped with a heated lid.
5. Place samples in a thermo cycler and start PCR.

Notes for the Components of the Reaction Mixture:

1. **Template DNA:** Usually the amount of template DNA is in the range of 0.01-1ng plasmid or phage DNA and 0.1-1ug for genomic DNA, for a total reaction mixture of 50ul.
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. **MgSO4 concentration:** Since Mg2+ ions form complex with dNTPs, primers and DNA templates, the optimal concentration of MgSO4 has to be selected for each experiment. In our experiments, at a final dNTP concentration of 200uM, 2mM MgSO4 concentration is suitable in most cases.
4. **dNTPs:** The final concentration of each dNTP in the reaction mixture is usually 200uM.
5. **Taq DNA polymerase:** Usually 1-1.5U of Taq DNA polymerase is used in the 50ul of reaction mix. Higher Taq DNA polymerase 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 Taq DNA polymerase (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 Taq DNA polymerase adds extra “A” nucleotides to the 3'-ends of PCR products.

