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Taq DNA Polymerase

Taq DNA Polymerase (5 U/μl)	100 μl
10×PCR Buffer (Mg2+ Plus)	1ml X2ea

For research use only

Cat No: YT1501 Size: 500 U Store at -20°C

Description

YTA Taq DNA Polymerase is a thermostable recombinant DNA polymerase resulting from thermophilic bacterium *Thermus* aquaticus. YTA Taq DNA Polymerase can amplify DNA target up to 5 kb (simple template). Its molecular weight is 94 kDa. The elongation velocity is 0.9~1.2kb/min (70~75°C).

It has 5' to 3' polymerase activity but lacks of 3' to 5' exonuclease activity that results in a 3'-dA overhangs PCR product.

Unit Definition

One unit is defined as the amount of the enzyme required to catalyze the incorporation of 10 nmole of dNTPs into an acid-insoluble form in 30 minutes at 70°C using hering sperm DNA as substrate.

Storage Buffer

20mM TrisCl (pH8.0), 100mM KCl, 3.2mM MgCl2 1mM DTT, 0.1% Triton X-100,0.1% Tween20, 0.2mg/ml BSA, 50% (v/v)glycerol

10X PCR Buffer with Mg2+

100mM Tris-HCl(PH 8.8), 500mMKCl, 1%Triton-X-100, 16Mm MgCl2

1. Add the following components to a sterile microcentrifuge tube sitting on ice:

1.1 Recommended PCR assay with PCR Buffer (Mg2+ plus)

Reagent	Quantity for 50µl volume	Final concentration
Sterile deionized water	Variable	-
10X PCR buffer (Mg2+ plus)	5 μ1	1X
dNTPs (10mM each)	1 μl	0.2 mM each
Primer I	Variable	0.4-1 μΜ
Primer II	Variable	0.4-1µM
Taq DNA	0.25-0.5 μ1	1.25-2.5 U/50µl
Polymerase(5U/ μl)	·	·
Template DNA	Variable	10pg-1µg
Total		50μ1

2. Mix contents of tube. Cap tubes and centrifuge briefly to collect the contents to the bottom.

When using a thermal cycler that does not contain a heated lid, overlay the reaction mixture with 25 µl mineral oil.

3. Perform 25-35 cycles of PCR amplification as follows:

Step	Temperature	Duration
Initial Denaturation	94°C	3 minutes
25-35 Cycles	94°C	30 seconds
	55-68°C 72°C	30 seconds 1 minutes
Final Extension	72°C	10 minutes

4. Incubate for an additional 10 min at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

5. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

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