FT-GX544A

Taq DNA Polymerases

Products Description

Description	Catalog #	Units	10x Reaction Buffer	50 mM $MgCl_2$
Taq DNA Polymerase (5U/µl)	GX544A	100	1 x 1,8 ml	1,5 ml
Taq DNA Polymerase (5U/µl)	GX544B	500	2 x 1,8 ml	1,5 ml
Red Taq DNA Polymerase (1U/µl)	DQJ54A	100	1 x 1,8 ml	1,5 ml
Red Taq DNA Polymerase (1U/µl)	DQJ54B	500	2 x 1,8 ml	1,5 ml
Green Taq DNA Polymerase (1U/µl)	LO3990	100	1 x 1,8 ml	1,5 ml
Green Taq DNA Polymerase (1U/µl)	LO3991	500	2 x 1,8 ml	1,5 ml

Storage: Store at -20°C for 24 months.

Introduction

Taq DNA Polymerase is a highly purified recombinant thermostable DNA polymerase that has been isolated from *E. coli* carrying a vector encoding the *Thermus aquaticus* DNA polymerase gene. The enzyme possesses a highly processive 5'→3' DNA polymerase activity with optimum activity achieved at 74°C.
It exhibits high thermal stability in withstanding prolonged incubations at elevated temperatures (95°C). Taq DNA Polymerase lacks 3'→5' exonuclease activity.

Red Taq DNA Polymerase contains an inert red dye which allows identification of the reactions which contain enzyme. The dye has no adverse effect on PCR.

Green reaction buffer contains two dyes (blue and yellow) that separate during electrophoresis to monitor migration progress. Reactions assembled with Green reaction buffer have sufficient density for direct loading onto agarose gels. Green reaction buffer is recommended for any amplification reaction that will be visualized by agarose gel electrophoresis and ethidium bromide staining. The dyes absorb between 225–300nm, making standard A260 readings to determine DNA concentration unreliable.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 74°C in the presence of the reaction buffer.

Enzyme Storage Buffer

20 mM Tris-HCl (pH 8.0 at 25 °C), 30 mM KCl, 0.1 mM DTT, 0.5% Tween 20, 50% (v/v) glycerol.

10x Reaction Buffer

670 mM Tris-HCl (pH 8.8 at 25 °C), 166 mM (NH4)2SO4, 4.5% Triton®-X-100, 2 mg/ml gelatine.

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5x Reaction Buffer

335 mM Tris-HCl (pH 8.8 at 25 °C), 83 mM (NH4)2SO4, 2.25% Triton®-X-100, 1 mg/ml gelatine.

Mg^{2+}-Solution

50 mM $MgCl_2$ (recommended final concentration: 1 – 4 mM).

Endodeoxyribonuclease Assay

No detectable conversion of covalently closed circular DNA to nicked DNA was observed after incubation of 10 units of Taq DNA Polymerase with 1 µg of digested DNA resp. 1µl of pBR322 DNA in 50 µl of Taq Buffer with KCL containing 1.5 mM MgCl2 for 4 hours at 37°C resp. 70°C.

Exodeoxyribonuclease Assay

No detectable degradation of lambda DNA/HindIII fragments was observed after incubation of 10 units of Taq DNA Polymerase with 1 µg of digested DNA resp. 1 µl of digested DNA in 50 µl of Taq Buffer with KCL containing 1.5 mM $MgCl_2$ for 4 hours at 37°C resp. 70°C.

Ribonuclease Assay

No detectable degradation of 28S/18S bands was observed after incubation of 10 units of Taq DNA Polymerase with 1 µg of total RNA in 50 µl of Taq Buffer with KCL containing 1.5 mM MgCl2 for 4 hours at 37°C resp. 70°C.

Functional Assay

0.1 ng of lambda DNA was amplified using specific primers to produce a distinct 500 bp band.

BASIC PROTOCOL

Mix the following components on ice in a thin-walled 0.2 ml PCR-tube:

Component	Volume (in µL)	Final concentration
10x Reaction Buffer	5	1x
50 mM MgCl2 solution	1.5	1.5 mM
12.5 mM dNTP-Mastermix	1	250 µM
Forward-Primer	variable	0.2 – 1 µM
Reverse-Primer	variable	0.2 – 1 µM
Template DNA	variable	< 1 µg
Red Taq DNA Polymerase (1 U/µl)	1.0 – 2.5	1 – 2.5 U
Taq DNA Polymerase (5 U/µl)	0.2 – 0.5	1 – 2.5 U
2x distilled, sterile water	Add to a final volume of 50	
Total volume	**50**	

CYCLING PROGRAM

Step	Temperature (in °C)	Time	Cycles
Initial activation	94	5 min	1
Denaturation	94	30 sec	23 – 35
Annealing	53*	45 sec	23 – 35
Extension	72	30 sec/kb	23 – 35
Final Extension	72	30 sec/kb	1

*: Approximately 5°C below Tm of primers

HINTS AND NOTES

- Mix the $MgCl_2$ solution before use by vortexing vigorously.
- Mix all components gently before use.
- Mix the components of the reaction gently after pipetting. No vortexing, no centrifugation.
- Keep the reaction tubes on ice as long as possible. Transfer the tubes from the ice to the cycler immediately after the denaturation temperature of about 94°C has been reached.

Reaction conditions (incubation temperatures and times, concentrations of template DNA, primers, magnesium ions and enzyme) depend on template and primers used. Optimal $MgCl_2$ concentrations vary between 1 – 4 mM and have to be determined empirically. However, many applications work at the standard concentration of 1.5 mM $MgCl_2$. Advanced applications on genomic DNA require higher $MgCl_2$ concentrations (2 – 3 mM) adjustable with the separate 50 mM MgCl2 solution supplied with the set.

OPTIMIZATION OF $MgCl_2$ CONCENTRATION IN REACTION MIXTURE

Final concentration of $MgCl_2$ in 50 µl reaction volume	Add 50 mM $MgCl_2$ solution
1.5 mM	1.5 µl
1.75 mM	1.75 µl
2.0 mM	2.0 µl
2.5 mM	2.5 µl
3.0 mM	3.0 µl
4.0 mM	4.0 µl

WARNING

For research only. Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

Related Products

- Agarose, regular uses, # 31272L
- TBE 10X, # UP86510A

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com.
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : Uptima / Interchim; Hotline : +33(0)4 70 03 73 06

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