TECHNICAL SHEET

PRODUCT NAME: **Kit Thermus aquaticus (Taq) polymerase, lyophilized (x5000 U)**

Code:	MT-E27TAQ
Physical State:	Lyophilized
Source:	*Escherichia coli.*
Description:	Recombinant Taq DNA polymerase expressed and purified from *Escherichia coli.*
Appearance:	Powder
Purity:	>95%
Storage Conditions:	Storage at room temperature.
Health & Safety Information:	Good Laboratory Practices should be followed when handling this material. The end user assumes all responsibility for care, custody and control of the material, including its disposal, in accordance with the respective national regulations.
Presentation:	Tris 20 mM Buffer with additives and salts, pH: 7.4

THIS PRODUCT IS INTENDED FOR RESEARCH USE ONLY.

Date: **17/08/2022**

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General description

This kit contains 5000 U of lyophilized *Thermus aquaticus* (Taq) DNA polymerase (MT-25TAQ). Certest Taq DNA polymerase is a thermally stable, processive, 5'→3' DNA polymerase, which exhibits more robust amplification than other commonly uses polymerases, showing very high activity over a wide range of PCR templates and routine molecular applications. It catalyzes the polymerization of nucleotides into duplex DNA in 5' → 3' direction and possesses a 5' → 3' exonuclease activity. The enzyme is isolated and purified from a plasmid expressed in *Escherichia coli* which contains the thermostable DNA polymerase gene of *Thermus aquaticus* cloned. The enzyme has a molecular weight of approximately 94kDa.

Package content

Reference	Description	Vials
MT-27TAQ	Thermus aquaticus (Taq) DNA polymerase, lyophilized (x5000 U)	1 x vial (clear)
MT-36REC	1X Reconstitution Buffer	0.5 mL; 1 x vial (clear)
MT-36MGCL	100 mM MgCl2 Buffer	1.5 mL; 1 x vial (blue)
MT-36TAQ	10X Taq Reaction Buffer	1.5 mL; 1 x vial (purple)

Storage

The kit is shipped at room temperature. Store at room temperature or below (until expiry date, see product label). Reconstituted Taq DNA polymerase must be stored at -20°C. Reconstitution instructions for the lyophilized enzyme are as it follows.

1) Centrifuge the vial of lyophilized enzyme at 12000 x g for 20 seconds.
2) For each tube containing 5000 U Taq DNA polymerase lyophilized, add 250 µL of 1X Reconstitution Buffer to reconstitute 20U/µL enzyme solution. Gently pipette up and down to dissolve the pellet/powder.
3) Place on ice, and aliquot into smaller volumes. Repeated freezing and thawing should be avoided.
4) For long-term storage, store at -20°C

Quality control

Each lot of Taq DNA polymerase is tested for sensitivity. Purity is also checked by SDS-electrophoresis and HPLC.

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Analytical Sensitivity Assay

Analytical sensitivity of each lot of TAQ DNA polymerase is evaluated performing standard curves in parallel with a reference lot. 10-fold serial dilution of control cDNA is performed and 5 µl of each dilution are added to 20 µl reaction mixtures containing TAQ DNA polymerase (10 U), specific primers and probe (500 nM and 250 nM respectively), 10 X Reaction Buffer (1 X), MgCl2 (3 mM) and dNTPs (0.8 mM each). Amplification conditions are those specify for Taq DNA Polymerase. Direct detection of PCR products is monitored by measuring the relative fluorescence units (RFU) produced by the result of probe hydrolysis after every cycle. And the resulting parallel standard curves are compared and assessed by analyzing the fluorescence, the minimum concentration of nucleic acids detection, Ct values and the sigmoid shape of the curves.

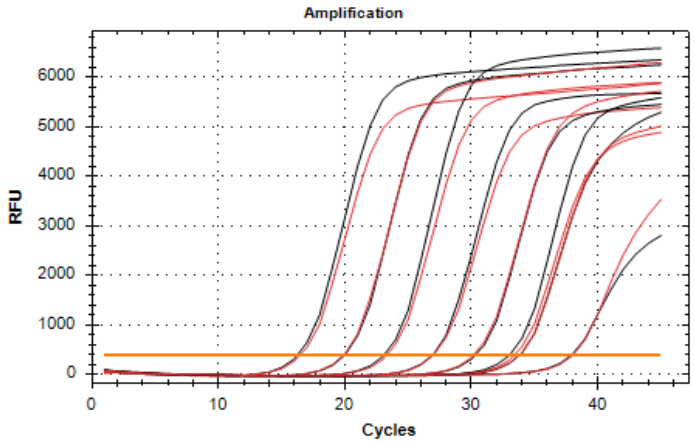
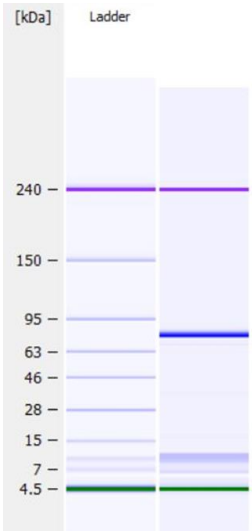


Image: comparation of parallel stand curves of a reference lot of Taq DNA polymerase (in black) and an evaluation lot of Taq DNA polymerase (in red). Similar efficiency of amplification is observed.

SDS-electrophoresis

SDS-PAGE gel in reducing conditions:



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Usage instructions

1) Prepare the following mix on ice and mix gently by pipetting.

Component	Volume	Final concentration (recommended)
10X Taq Reaction Buffer	2 µL	1X
Primers	variable	250 – 500 nM
dNTPs Mix	variable	2 mM (0.5 mM of each)
100 mM $MgCl_2$ Buffer	variable	1.5 mM-5mM
Taq DNA polymerase	0.5 µL	10 U
DNA template	variable	
Nuclease free water	Adjust to 20 µL	

2) Program the cycler according to the manufacturer´s instructions. Each program should start with an initial denaturation step at 95°C for 2- 5 minutes. Recommended elongation time is 1 min per 1 kb of target. For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair. Example: 1X (95°C 2 minutes) + 45X (95°C 10 seconds + 60°C 20 seconds + 72°C 90 seconds).

If technical support is needed, please contact us (bioscience@certest.es).

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