diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME MethylTaq DNA polymerase	
Cat. No: AF-103-0250	**Format:** 250 units

Description

MethylTaq DNA polymerase is a high-performance Hot Start thermostable recombinant DNA polymerase. MethylTaq is an extremely robust modified Taq DNA polymerase that completely lacks any activity below 74°C thus avoiding non-specific priming at low temperature. This highly robust enzyme produces excellent results in demanding applications and it is recommended for PCR after hMeDIP (Cat. No. AF-104-0016), MeDIP, after Bisulfite Conversion (Cat. No. AF-106-0024) and for use with hmdCTPs (Cat. No. AF-102-0300).

MethylTaq DNA polymerase requires a 10 minutes activation step at 95°C to reach maximal initial activity.

Content:

1 tube of 250 Units MethylTaq polymerase
1 tube of 1.5 ml of 10 X MethylTaq polymerase buffer
1 tube of 1.5 ml of 25 mM MgCl2

Features:

- Reduces non-specific PCR product formation
- Hot start DNA polymerase for robust amplification
- Optimized for use with difficult to amplify DNA (e.g. bisulfite-treated DNA, ...)
- Easy of use
- Processes fragments of up to 5Kb
- Products suitable for TA cloning

Applications:

- Demanding applications such as PCR amplification after MeDIP or hMeDIP
- PCR amplification and cloning after Bisulfite Conversion
- PCR amplification using modified nucleotides such as hydroxymethylated dCTPs (Cat. No. AF-102-0300)

Quality control

Each lot is tested for the absence of nicking and priming activities, exonucleases and non-specific endonucleases.

Source

Purified from an *E. coli* strain, which carries a Thermus species DNA polymerase overexpressing plasmid.

Shipping conditions

Shipment at ambient temperature has no detrimental effect on the performance of this enzyme.

Storage & Dilution buffer:

Storage at -20°C is recommended.
20 mM Tris HCl (pH8.0), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 50 % glycerol, stabilizers.

Analysis conditions:

25 mM TAPS, pH 9.3 (at 25°C); 50 mM KCl; 2 mM $MgCl_2$; 1 mM ß-mercaptoethanol; 250 µM each dCTP, dGTP, dTTP : 250 µM (3H) dATP (0.05 Ci/mmol); activated salmon sperm DNA (1.25 µg/µl); total volume of 50 µl.

Unit definition
One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid- insoluble form in 30 minutes at 72°C under the analysis conditions.

Reaction Conditions
Thaw reagents completely and put them on ice. Mix all reagents well before use.

For a 50 µl reaction:
MethylTaq 10x buffer : 5 µl
dNTPs (5 mM stock solution): 2 µl (200 µM final concentration).
DNA template: 1 - 20 ng
Primers : 0.1 nmol
$MgCl_2$: 4 µl (2 mM, see below)
MethylTaq DNA polymerase (5 U/µl): 1 unit to 2.5 units

$MgCl_2$ (25 mM) : MethylTaq is a magnesium-dependent enzyme. The recommended magnesium concentration varies from 1 to 4 mM.
H_2O : up to 50µl

Cycling conditions (hMeDIP)		
Step	Conditions	Cycles
MethylTaq DNA polymerase activation	10 min at 95°C	1x
Denaturation	30 sec at 95°C	30 - 40x
Annealing	temperature depends on primer Tm	
Elongation	1 minute/kb at 72°C (suggested)	
	hold at 4°C	1x

Cycling conditions (Bisulfite-treated DNA)		
Step	Conditions	Cycles
MethylTaq DNA polymerase activation	10 min at 95°C	1x
Denaturation	30 sec at 95°C	40x
Annealing	1 min, temperature depends on Primer Tm	
Elongation	1 min at 72°C	
Final extension	5 min at 72°C	1x
	hold at 20°C	1x

Important:
No amplification will be observed without the 10 minutes activation step at 95°C.
Time and temperature for denaturation and annealing steps depend on the type of machine and primers. We advise that you check primer design using primer design software. This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimisation.

For research or laboratory use only, not for diagnostics or therapeutics use.