EpiMark™ Hot Start *Taq* DNA Polymerase

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M0490S

100 rxns (50 µl vol) **5,000 U/ml** **Lot: 0031112**
RECOMBINANT **Store at –20°C** **Exp: 12/13**

Description: EpiMark™ Hot Start *Taq* DNA Polymerase is a mixture of *Taq* DNA Polymerase and a temperature sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 45°C, but releases the enzyme during normal PCR cycling conditions. This permits assembly of PCR reactions at room temperature. An advantage of the aptamer-based hot start mechanism is that it does not require a separate high temperature incubation step to activate the enzyme. The advanced aptamer-based hot-start activity coupled with the supplied optimized reaction buffer makes the EpiMark Hot Start *Taq* DNA Polymerase an excellent choice for use on bisulfite-converted DNA.

Taq DNA polymerase possesses a 5´- 3´ polymerase activity (1,2,3) and a 5´ flap endonuclease activity (4,5).

EpiMark Hot Start *Taq* DNA Polymerase is supplied with 5X EpiMark Hot Start *Taq* Reaction Buffer, providing robust amplification for bisulfite-converted DNA and AT-rich amplicons.

Source: An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1

Applications:

- PCR from bisulfite-converted DNA

Supplied in: 100 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM dithiothreitol, 0.5% Tween 20, 0.5% IGEPAL® CA-630 and 50% glycerol

Reagents Supplied with Enzyme:
5X EpiMark Hot Start *Taq* Reaction Buffer

Reaction Conditions: 1X EpiMark Hot Start *Taq* Reaction Buffer, bisulfite-converted DNA template, primers, 200 µM dNTPs (not included) and 1.25 units of EpiMark Hot Start *Taq* DNA Polymerase in a total reaction volume of 50 µl.

1X EpiMark Hot Start *Taq* Reaction Buffer:
20 mM Tris-HCl
1.8 mM $MgCl_2$
22 mM NH_4Cl
22 mM KCl
0.06% IGEPAL CA-630
0.05% Tween 20
pH 8.9 at 25°C

Unit Definition: One unit is defined as the amount of enzyme that will incorporate 15 nmol dNTP into acid insoluble material in 30 minutes at 75°C.

Unit Assay Conditions: 1X ThermoPol Reaction Buffer, 200 µM dNTPs including [^{3}H]-dTTP and 200 µg/ml activated Calf Thymus DNA.

Heat Inactivation: No

Quality Control Assays

High Sensitivity PCR Assay: 30 cycles of PCR amplification of 10 pg Lambda with 0.625 units of EpiMark Hot Start *Taq* DNA Polymerase in a 25 µl reaction containing 50 ng human genomic DNA, 200 µM dNTPs, 0.2 µM primer and 1X EpiMark Hot Start *Taq* Reaction Buffer resulted in a significant, enzyme-dependent increase in yield of the 2 kb Lambda product as compared to a standard PCR reaction. In non-hot start PCR control reactions, characteristic non-specific bands are produced from this set of primers and template.

3´→ 5´ Exonuclease Activity: Incubation of a 20 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 10 nM fluorescent internally labeled oligonucleotide for 30 minutes at either 37°C or 75°C yields no detectable 3´→ 5´ degradation as determined by capillary electrophoresis.

Endonuclease Activity: Incubation of a 50 µl reaction in ThermoPol Reaction Buffer containing a minimum of 20 units of *Taq* DNA Polymerase with 1 µg of supercoiled ϕX174 DNA for 4 hours at 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

Inhibition Assay: Greater than 95% inhibition is observed after a 16 hour incubation at 25°C in a 50 µl primer extension assay containing 2.5 units of EpiMark Hot Start *Taq* DNA Polymerase in 1X ThermoPol Reaction Buffer with 200 µM dNTPs including [^{3}H]-dTTP and 15 nM primed single-stranded M13mp18.

PCR

The Polymerase Chain Reaction (PCR) is a powerful and sensitive technique for DNA amplification (6). *Taq* DNA Polymerase is an enzyme widely used in PCR (7). EpiMark Hot Start *Taq* DNA Polymerase allows for greater PCR sensitivity and permits room temperature PCR reaction set-up. The following guidelines are provided to ensure successful PCR using New England Biolabs' EpiMark Hot Start *Taq* DNA Polymerase. These guidelines cover routine PCR reactions.

Reaction setup:
Due to the hot-start nature of the enzyme, reactions can be assembled on the bench at **room temperature** and transferred to a thermocycler. *No separate activation step is required to release the inhibitor from the enzyme.*

Add to a sterile thin-walled PCR tube:

COMPONENT	25 µl REACTION	50 µl REACTION	FINAL CONCENTRATION
5X EpiMark Hot Start *Taq* Reaction Buffer	5 µl	10 µl	1X
10 mM dNTPs	0.5 µl	1 µl	200 µM
10 µM Forward Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
10 µM Reverse Primer	0.5 µl	1 µl	0.2 µM (0.05–1 µM)
EpiMark Hot Start *Taq* DNA Polymerase	0.125 µl	0.25 µl	1.25 units/ 50 µl PCR
Template DNA	variable	variable	<1,000 ng
Nuclease-Free Water	to 25 µl	to 50 µl	

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Transfer PCR tubes to a cycler and begin thermocycling:

Thermocycling Conditions for a Routine PCR:

STEP	TEMP	TIME
Initial Denaturation	95°C	30 seconds
35–40 Cycles	95°C	15–30 seconds
	45–68°C	15–60 seconds
	68°C	1 minute/kb
Final Extension	68°C	5 minutes
Hold	4–10°C	

General Guidelines:

1. Template:
   Use of high quality, bisulfite-treated DNA templates greatly enhances the success of PCR reactions. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	AMOUNT
Genomic	1 ng–1 µg
Plasmid or Viral	1 pg–1 ng

2. Primers:
   Oligonucleotide primers for bisulfite-converted DNA are generally 25–40 nucleotides in length, and should not contain CpG sites within their sequence to ensure unbiased amplification of both methylated or unmethylated DNA. Primer design and analysis can be facilitated using programs such as MethPrimer (http://www.urogene.org/methprimer/index1.html) or BiSearch (http://bisearch.enzim.hu). methBLAST (http://medgenugent.be/methBLAST) allows you to check your primers or bisulfite-converted DNA by blasting them against the unmethylated and methylated genomic sequences of human, mouse and rat. The recommended T_m for a primer set can be determined using the New England Biolabs T_m calculator (available at www.neb.com). The final concentration of each primer in a PCR reaction may be 0.05–1 µM, typically 0.2 µM.

3. Mg^{++} and additives:
   Mg^{++} concentration of 1.5–2.0 mM is optimal for most PCR products generated with EpiMark Hot Start *Taq* DNA Polymerase. The final Mg^{++} concentration in 1X EpiMark Hot Start *Taq* Reaction Buffer is 1.8 mM. This supports satisfactory amplification of most amplicons. However, Mg^{++} can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$ (sold separately).

4. Deoxynucleotides:
   The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. EpiMark Hot Start *Taq* DNA Polymerase Concentration:
   We generally recommend using EpiMark Hot Start *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of EpiMark Hot Start *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

(see other side)

CERTIFICATE OF ANALYSIS

6. Denaturation:
   No separate activation step is required to release the hot start inhibitor from the enzyme. An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. Longer denaturation times can further damage bisulfite-converted DNA and is not recommended.

   During thermocycling a 15–30 second denaturation at 95°C is recommended.

7. Annealing:
   The annealing step is typically 15–60 seconds. The annealing temperature is based on the T_m of the primer pair and is typically 45–68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5°C below the calculated T_m.

8. Extension:
   The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C is recommended.

9. Cycle number:
   Generally, 35–40 cycles yields sufficient product. Up to 45 cycles may be required to detect low copy number targets from bisulfite-converted DNA.

10. PCR product:
    The PCR products generated using EpiMark Hot Start *Taq* DNA Polymerase contain dA overhangs at the 3´ end; therefore the PCR products can be ligated to dT/dU-overhang vectors.

References:

1. Chien, A., Edgar, D.B. and Trela, J.M. (1976) *J. Bact.*, 127, 1550–1557.
2. Kaledin, A.S., Slyusarenko, A.G. and Gorodetskii, S.I. (1980) *Biokhimiya*, 45, 644–651.
3. Lawyer, F.C. et al. (1993) *PCR Methods and Appl.*, 2, 275–287.
4. Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990) *Nucleic Acids Res.*, 18, 7317–7322.
5. Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993) *Science,* 260, 778–783.
6. Saiki R.K. et al. (1985) *Science,* 230, 1350–1354.
7. Powell, L.M. et al. (1987) *Cell,* 50, 831–840.
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Companion Products Sold Separately:

Diluent F
#B8006S 4.0 ml

EpiMark™ Hot Start *Taq* Reaction Buffer Pack
#B0490S 8.0 ml

EpiMark™ Bisulfite Conversion Kit
#E3318S 48 reactions

Magnesium Chloride ($MgCl_2$) Solution
#B9021S 6.0 ml

Deoxynucleotide Solution Set
#N0446S 25 µmol each

Deoxynucleotide Solution Mix
#N0447S 8 µmol each
#N0447L 40 µmol each

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