Hemo KlenTaq®

1-800-632-7799
info@neb.com
www.neb.com

M0332S 015120614061

M0332S

200 reactions (25 µl vol) **Lot: 0151206**

RECOMBINANT **Store at –20°C** **Exp: 6/14**

Description: Hemo KlenTaq is a truncated version of *Taq* DNA Polymerase, lacking the first 280 amino acids (1). Hemo KlenTaq also contains mutations that make it resistant to inhibitors present in whole blood. It can amplify directly from whole blood from humans and mice. Hemo KlenTaq tolerates up to 10% whole blood in a 25 µl reaction (20% in 50 µl reaction).

Source: An *E. coli* strain that carries a mutant *Taq* DNA polymerase gene. The protein lacks the N-terminal 5´→ 3´ exonuclease domain and the gene has three internal point mutations.

Applications:
- Whole Blood PCR
- Primer extension

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 Hemo KlenTaq Reaction Buffer

Reaction Conditions: 1X Hemo KlenTaq Reaction Buffer, DNA template, 0.3 µM primers, 200 µM dNTPs and 2 µl of Hemo KlenTaq DNA Polymerase in a total reaction volume of 25 µl.

1X Hemo KlenTaq Reaction Buffer:
60 mM Tricine
5 mM $(NH_4)_2SO_4$
3.5 mM $MgCl_2$
6% glycerol
pH 8.7 @ 25°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

0.5 kb Whole Blood PCR: 35 cycles of PCR amplification of 10% whole blood treated with sodium heparin, sodium EDTA, potassium EDTA or sodium citrate in a 50 µl reaction with 4 µl of Hemo KlenTaq in the presence of 200 µM dNTPS, 0.3 µM primers and 1X Hemo KlenTaq Reaction Buffer results in the expected 0.5 kb product.

3´→ 5´ Exonuclease Activity: Incubation of a 20 µl reaction in Hemo KlenTaq Reaction Buffer containing a minimum of 400 units of Hemo KlenTaq 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 Hemo KlenTaq Reaction Buffer containing a minimum of 400 units of Hemo KlenTaq with 1 µg of supercoiled ϕX174 DNA for 4 hours at either 37°C or 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

PCR Guidelines
The following guidelines are provided to ensure successful PCR using New England Biolabs' Hemo KlenTaq DNA Polymerase.

Protocol:
Before use, thoroughly mix 5X Hemo KlenTaq Reaction Buffer by inversion.

1. Add to a thin-walled PCR tube on ice:

Component	25 µl	50 µl	Final Concentration
5X Hemo KlenTaq Reaction Buffer	5 µl	10 µl	1X
10 mM dNTP	0.5 µl	1 µl	0.2 mM
Hemo KlenTaq	2 µl	4 µl	
10 µM Forward Primer	0.75 µl	1.5 µl	0.3 µM (0.05–1 µM)
10 µM Reverse Primer	0.75 µl	1.5 µl	0.3 µM (0.05–1 µM)
Whole Blood	up to 2.5 µl*	up to 10 µl**	
Nuclease-free Water	to 25 µl	to 50 µl	

2. Before adding blood, thoroughly mix components by pipetting up and down.
3. Add blood last and let sink to the bottom of the tube.

(see other side)

CERTIFICATE OF ANALYSIS

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Heat Inactivation: No

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0.5 kb Whole Blood PCR: 35 cycles of PCR amplification of 10% whole blood treated with sodium heparin, sodium EDTA, potassium EDTA or sodium citrate in a 50 µl reaction with 4 µl of Hemo KlenTaq in the presence of 200 µM dNTPS, 0.3 µM primers and 1X Hemo KlenTaq Reaction Buffer results in the expected 0.5 kb product.

3´→ 5´ Exonuclease Activity: Incubation of a 20 µl reaction in Hemo KlenTaq Reaction Buffer containing a minimum of 400 units of Hemo KlenTaq 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 Hemo KlenTaq Reaction Buffer containing a minimum of 400 units of Hemo KlenTaq with 1 µg of supercoiled ϕX174 DNA for 4 hours at either 37°C or 75°C results in < 10% conversion to the nicked form as determined by agarose gel electrophoresis.

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Hemo KlenTaq	2 µl	4 µl	
10 µM Forward Primer	0.75 µl	1.5 µl	0.3 µM (0.05–1 µM)
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Whole Blood	up to 2.5 µl*	up to 10 µl**	
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2. Before adding blood, thoroughly mix components by pipetting up and down.
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(see other side)

CERTIFICATE OF ANALYSIS

4. Overlay the sample with mineral oil if using a PCR machine without a heated lid.
5. Transfer PCR tubes to a PCR machine with a preheated block at 95°C and start cycling.
6. For reactions containing ≥ 10% blood, a 50 µl reaction volume is recommended.

DNA Template: Whole blood samples treated with sodium heparin, sodium EDTA, potassium EDTA, or sodium citrate are recommended. Although up to 20% blood (v/v) can be used in the PCR reactions, 5%–10% is recommended. High concentrations of blood are not recommended due to difficulties in recovery of the aqueous supernatant from blood cell debris that remains after the reaction. Dry blood stored on Guthrie cards or 903 cards (Whatman, NJ) can be used directly by adding 1 mm punch disc to a 25 µl PCR reaction. If blood is stored on FTA paper (Whatman, NJ), incubating the 1 mm punch disc first in 50 µl water at 50°C for 5 minutes is recommended. Discard the 50 µl water and use the disc directly in a 25–50 µl PCR reaction. For GC-rich targets, up to 10% DMSO can be included (2).

Primers: Primers are generally 20–30 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as PrimerSelect™ (DNAStar Inc, Madison, MI) and Primer3 (http://frodo.wi.mit.edu/primer3) can be used to design or analyze primers.

The final concentration of each primer in a typical PCR reaction is 0.05–1 µM, ideally 0.3 µM.

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

Starting Reactions: Hemo KlenTaq has increased cold sensitivity, conferring some hot start character on the enzyme (1). Nonspecific priming can be minimized by assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (95°C).

Denaturation Temperature and Duration: An initial 3 minute denaturation step at 95°C is recommended prior to PCR cycling to fully lyse the blood cells and release/denature the DNA.

Annealing Temperature and Duration: The annealing step is typically 30 seconds to 1 minute.

The annealing temperature can be optimized by doing a temperature gradient PCR starting 5°C below the calculated melting temperature (T_m). We recommend using NEB's T_m Calculator, available at www.neb.com/TmCalculator to determine appropriate annealing temperatures for PCR.

Extension Time: The recommended extension temperature is 68°C. A final extension of 10 minutes at 68°C is recommended. The extension rate is generally 2 minutes per kb.

Cycling Conditions: Generally, 30–40 cycles give optimal amplification.

Initial denaturation:	95°C	3 minutes
30–40 cycles	95°C	20 seconds
	50–68°C	30 seconds
	68°C	2 minutes per kb
Final extension:	68°C	10 minutes
Hold:	4°C	

References:

1. Kermekchiev, M.B., et al. (2009) *Nucleic Acids Res.,* 37, e40.
2. Sun, Y., Hegamyer, G. and Colburn, N. (1993) *Biotechniques,* 15, 372–374.

Companion Products:

Hemo KlenTaq Reaction Buffer	
#B0332S	6.0 ml
Diluent F	
#B8006S	6.0 ml
Deoxynucleotide Solution Set	
#N0446S	25 µmol of each
Deoxynucleotide Solution Mix	
#N0447S	8 µmol each
#N0447L	40 µmol each

4. Overlay the sample with mineral oil if using a PCR machine without a heated lid.
5. Transfer PCR tubes to a PCR machine with a preheated block at 95°C and start cycling.
6. For reactions containing ≥ 10% blood, a 50 µl reaction volume is recommended.

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Companion Products:

Hemo KlenTaq Reaction Buffer	
#B0332S	6.0 ml
Diluent F	
#B8006S	6.0 ml
Deoxynucleotide Solution Set	
#N0446S	25 µmol of each
Deoxynucleotide Solution Mix	
#N0447S	8 µmol each
#N0447L	40 µmol each

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