Traferogen [For research use only]

Product Name: TGHS (TG Hot-Start) Taq DNA polymerases

Product Overview

Enzyme	Hot-start Taq
Origin	Recombinant Taq & a thermolabile inhibitor
3'-5' proof reading activity	No
Mis-incorporation rate	$< 10^{-4}$
Primer extension @ RT	Inhibited
Applications	Non-specific primer extension and primer dimerization at temperature lower than 55°C by Taq DNA polymerase are inhibited. Taq activity can be heat- activated at 95°C for 2min. • Routine PCR (<5kb) • Single step reverse transcription PCR, • Sybr Green-based qPCR • Genotyping (for mice, zebrafish, drosophila, sea urchins etc.), • Others.
Store @	-20°C for 1 year
Activity	5U/μl
Catalog #	**EH316-1000U**

[Certificate of Analysis] Enzymatic activities of Taq and Hot-Start Taq were tested in amplification of a 4.6 kb DNA fragment of a single copy gene of mouse genomic DNA. A 15 kb mouse genomic fragment was amplified by TP polymerase for QC.

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Table 1. PCR reaction setup #1.

Component	Volume (µl)/ 50 µl reaction	Final concentration/amount
DNA template	X	0.1pg-100ng for plasmid or cDNA; 5pg-100ng for genomic DNA.
10X HF or HP buffer	5	1X
dNTPs (2.5mM each)	4-8	200-400 µM
Forward Primer (10 µM)	0.5-2.5	0.1-0.5 µM
Reverse Primer (10 µM)	0.5-2.5	0.1-0.5 µM
$MgCl_2$ (25mM)*	0-1.0	0.1-0.5 mM
DMSO (optional)	0-5	1-10% w/v
5X Enhancer (optional)	0-10	1X
Taq, or Hot-start Taq, or TP DNA polymerase (5U/µl)§	0.5-1	2.5-5U/50 µl rxn
H_2O	to 50 µl	

*Optimal [Mg^{++}] needs to be titrated. § for amplicon < 5 kb, either Taq or Hot-start Taq is effective; for amplification of fragment larger than 5 kb, TP DNA polymerase is recommended.

Table 2. PCR reaction setup #2.

Component	Volume (µl)/ 50 µl reaction	Final concentration/amount
DNA template	X	0.1pg-100ng for plasmid or cDNA; 5pg-100ng for genomic DNA.
10X FD or FB buffer	5	1X
dNTPs (2.5mM each)	4-8	200-400 µM
Forward Primer (10 µM)	0.5-2.5	0.1-0.5 µM
Reverse Primer (10 µM)	0.5-2.5	0.1-0.5 µM
Taq, Hot-start Taq or TP DNA polymerase (5U/µl) §	0.5-1	2.5-5U/50 µl rxn
H_2O	to 50 µl	

Table 3. Thermal cycling protocol (< 5Kb)

Steps	Temperature & Duration	Cycle number
Initial Denature	95°C, 2 min	1
Amplification • Denature • Annealing • elongation	95°C, 20 sec Tm-5°C, 30 sec 72°C, N* x 45 sec/kb	35-40
Final extension	72°C, 2-5min	1
Holding	10-4°C, ∞	

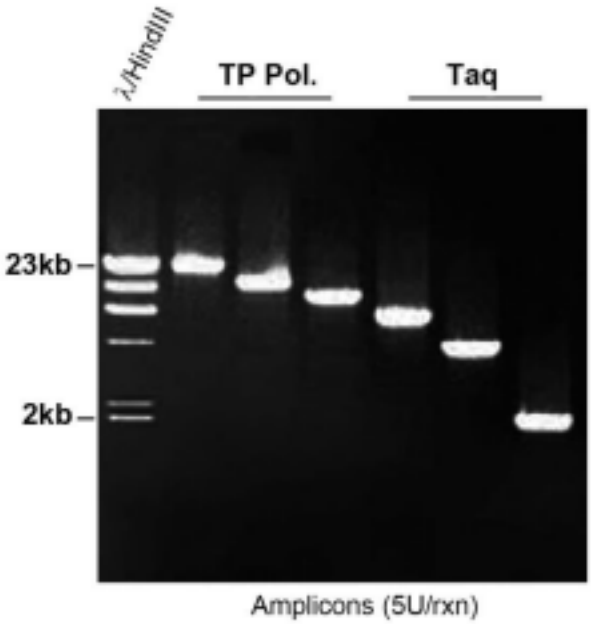
* N is the expected amplicon length in Kb.

Table 4. Thermal cycling protocol (5-30Kb) §

Steps	Temperature & Duration	Cycle number

Initial Denature	92°C, 2 min	1
Amplification • Denature • Annealing • elongation	92°C, 15 sec Tm-5°C, 30 sec 68°C, N* x 1 min/kb	10
Amplification • Denature • Annealing • elongation	92°C, 15 sec Tm-5°C, 30 sec 68°C, N* x 1 min/kb + 15 sec increment/addition cycle	25-28
Final extension	68°C, 5min	1
Holding	10-4°C, ∞	

* N is the expected amplicon length in Kb. § for long template PCR amplification, primers consisting of 25-34 nucleotides with Tm=62-68°C were preferred.



QC validation of Taq and TP DNA polymerase. Primers sets were used to amplify DNA fragments of various lengths from mouse β-actin locus.