

Thermo Scientific Thermo-Start Taq DNA Polymerase

Thermo-StartTM Taq DNA Polymerase is a chemically modified **Description:**

version of ThermoPrime *Taq* DNA Polymerase. It is completely inactive at room temperature, preventing the formation and subsequent amplification of non-specific products. The enzyme requires an

activation step at 95°C for 15 minutes.

Enzyme Source: Thermus aquaticus

Concentration: 5 units/µl

Unit Definition: One unit of enzyme is defined as the amount that will incorporate

10nmoles of dNTPs into acid insoluble material in 30 minutes at 74°C

under the analysis conditions below.

Thermo-Start TM has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading). **Associated**

Activities:

Store Thermo-StartTM Taq DNA Polymerase at -20°C, in a constant Storage

temperature freezer for up to 12 months. Shipped on ice within the UK **Conditions:**

and on dry ice for international and within the US.

Kit Contents

Vial	Pack Size (cap color)		
Viai	A	В	С
Thermo-Start Taq DNA Pol.	1 x 50μ1	10 x 50μl	20 x 50μ1
	(clear)	(clear)	(clear)
Thermo-Start PCR Buffer with MgCl ₂	1 x 1.25ml	10 x 1.25ml	20 x 1.25ml
	(purple)	(purple)	(purple)



Example of Protocol:

Mix and spin down the solutions prior to use

	Volume	Final Concentration 1X	
Thermo-Start Taq	0.125µl	0.625 U	
DNA Pol. (5U/μl)	0.125μ1 0.625 0		
10X Thermo-Start PCR Buffer	2.5µl 1X (+1.5mM MgCl ₂)		
+ 15mM MgCl ₂	2.5µl	TX (+1.5IIIWI WIGCI ₂)	
dNTP Mix (20mM)	1µl	0.5mM of each nucleotide	
Primer forward (10µM each)	1.25µl*	0.5μM*	
Primer reverse (10µM each)	1.25µl*	0.5μM*	
Water (PCR Grade)	variable		
DNA Template	0.5 - 10µl	0.5 - 125ng	
Total Volume	25 µl		

^{*}Scale up or down the volume and concentration as appropriate

Note: These recommendations are intended as basic guidelines.

Example of Program:

	Temp.	Time	Number of cycle
Initial Denaturation	95°C	15 min	1 cycle
Denaturation	95°C	20 sec	20 +- 40
Annealing	50-65°C	30 sec	30 to 40 cycles
Extension**	72°C	60 sec	Cycles
Final Extension	72°C	5 min	1 cycle

^{**}Increase length of time in proportion to size of amplicon, Taq DNA Polymerase extends at approximately 1000 bp/min.

Incremental Activation:

For extra stringency, the enzyme can be activated gradually during the PCR in a series of steps. The initial activation step is replaced by longer (2 minutes) denaturation steps for the first 7–8 cycles of the reaction.



Analysis 25mM Conditions: 50mM TAPS, pH 9.3 (at 25°C)

[tris-(hydroxymethyl)-methyl-amino-propane sulfonic acid, sodium salt]

 $\begin{array}{ccc} 50 mM & KCl \\ 2 mM & MgCl_2 \end{array}$

1mM β-mercaptoethanol

 $\begin{array}{lll} 200\mu M & \text{of each: dATP, dGTP, dTTP} \\ 100\mu M & [\alpha^{32}P]\text{-dCTP } (0.05 \text{ to } 0.1 \text{ Ci/mmol}) \\ 1.25\mu g/\mu l & \text{activated salmon sperm DNA} \end{array}$

Water added to a total volume of $50\mu l$. Incubated at $74^{\circ}C$ for 10 minutes. The enzyme is first treated with a 15 minute activation step at 95°C. The amount of incorporated dNTPs is determined by trichloroacetic acid precipitation.

Storage Buffer:

100mM KCl

20mM Tris-HCl, pH 9.2 (at 25°C)

0.1mM EDTA (ethylenediaminetetraacetic acid)

1mM DTT (dithiothreitol)

0.5% Tween® 20 0.5% Nonidet® P40 50% (v/v) Glycerol

Ordering Information:

AB-1908/A	Thermo-Start Taq DNA Polymerase	250 units
AB-1908/B	Thermo-Start Taq DNA Polymerase	10 x 250 units
AB-1908/C	Thermo-Start Taq DNA Polymerase	100 x 250 units

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