

Thermo Scientific Thermo-Start *Taq* DNA Polymerase with High Performance Buffer

Description: Thermo-Start™ *Taq* DNA Polymerase is a chemically modified 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.

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

Storage Conditions: Store Thermo-Start™ *Taq* DNA Polymerase at -20°C, in a constant temperature freezer for up to 12 months. Shipped on ice within the UK and on dry ice for international and within the US.

Kit Contents

Vial	Pack Size (cap color)		
	A	B	C
Thermo-Start <i>Taq</i> DNA Pol.	1 x 50μl (clear)	10 x 50μl (clear)	100 x 50μl (clear)
Thermo-Start High Performance PCR Buffer	1 x 1.25ml (red)	10 x 1.25ml (red)	100 x 1.25ml (red)
MgCl ₂	1 x 1.5ml (clear)	10 x 1.5ml (clear)	100 x 1.5ml (clear)

Example of Protocol:

Mix and spin down the solutions prior to use

	Volume	Final Concentration 1X
Thermo-Start Taq DNA Pol. (5U/ μ l)	0.125 μ l	0.625 U
10X Thermo-Start HP Buffer	2.5 μ l	1X
dNTP Mix (20mM)	1 μ l	0.5mM of each nucleotide
MgCl ₂ (25mM)	1.5 μ l*	1.5mM*
Primer forward (10 μ M each)	1.25 μ l*	0.5 μ M*
Primer reverse (10 μ M each)	1.25 μ l*	0.5 μ M*
Water (PCR Grade)	variable	
DNA Template	0.5 - 10 μ l	0.5 - 125ng
Total Volume	25 μ l	

*Scale up or down the volume and concentration as appropriate

Note: These recommendations are intended as basic guidelines. Magnesium chloride concentration and amount of enzyme should be optimized according to template and primer combination.

Example of Program:

	Temp.	Time	Number of cycle
Initial Denaturation	95°C	15 min	1 cycle
Denaturation	95°C	20 sec	30 to 40 cycles
Annealing	50-65°C	30 sec	
Extension**	72°C	60 sec	
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	TAPS, pH 9.3 (at 25°C)
Conditions:	50mM	[tris-(hydroxymethyl)-methyl-amino-propane sulfonic acid, sodium salt]
	2mM	KCl
	1mM	MgCl ₂
	200µM	β-mercaptoethanol
	100µM	of each: dATP, dGTP, dTTP
	1.25µg/µl	[α- ³² P]-dCTP (0.05 to 0.1 Ci/mmol)
		activated salmon sperm DNA

Water added to a total volume of 50µl. Incubated at 74°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-1057/A	Thermo-Start <i>Taq</i> DNA Polymerase with High Performance Buffer	250 units
	AB-1057/B	Thermo-Start <i>Taq</i> DNA Polymerase with High Performance Buffer	10 x 250 units
	AB-1057/C	Thermo-Start <i>Taq</i> DNA Polymerase with High Performance Buffer	100 x 250 units

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