

Thermo-Start™ PCR Kit

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 (cap color)	Pack Size	
	A	B
Thermo-Start <i>Taq</i> DNA Pol. (clear)	1 x 50µl	10 x 50µl
Thermo-Start PCR Buffer (yellow)	1 x 1.25ml	10 x 1.25ml
MgCl ₂ (clear)	1 x 1.5ml	10 x 1.5ml
dNTP Mix (brown)*	0.5ml	3 x 1.6ml

*contains 5mM of each dNTP (dATP, dGTP, dCTP and dTTP)

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Example of Protocol:

Mix and spin down the solutions prior to use

	Volume	Final Concentration 1X
Thermo-Start Taq DNA Pol. (5U/μl)	0.125μl	0.625 U
10X Thermo-Start PCR Buffer	2.5μl	1X
dNTP Mix (20mM)	1μl	0.2mM of each nucleotide
MgCl ₂ (25mM)	1.5μl*	1.5mM*
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. 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 Conditions:	25mM	TAPS, pH 9.3 (at 25°C)
	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-0908/A/N	Thermo-Start PCR Kit	250 units
	AB-0908/B/N	Thermo-Start PCR Kit	2500 units

All sizes are supplied with 10X Reaction Buffer, 20mM dNTP Mix and 25mM MgCl₂.

Troubleshooting

For troubleshooting, see www.abgene.com/troubleshoot.asp or contact Thermo Fisher Scientific (ABgene) TechSupport at abgene.techsupport@thermofisher.com

UK TechSupport, call +44 (0) 1372 840 410

For all other regions, please contact your local Thermo Fisher Scientific (ABgene) office / distributor.

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