

Thermo-Start™ with 10X ReddyMix™ PCR 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**.

ReddyMix™ PCR Buffer has an inert red tracker dye and a precipitant added. After thermal cycling a sample (10–30%) of the PCR mix may be loaded directly onto an agarose gel without the addition of gel loading buffer. The dye migrates between bromophenol blue and xylene cyanol at approximately 300bp, depending on agarose concentration.

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).

Kit Contents

Vial (cap color)	Pack Size	
	A	B
Thermo-Start <i>Taq</i> DNA Pol. (clear)	50μl	10 x 50μl
Thermo-Start ReddyMix™ Buffer (yellow)	1.25ml	10 x 1.25ml
MgCl ₂ (clear)	1.25ml	10 x 1.25ml

For Research Purposes Only

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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.

Example of Protocol:

Mix and spin down the solutions prior to use

	Volume	Final Concentration 1X
Thermo-Start <i>Taq</i> DNA Pol. (5U/μl)	0.125μl	0.625 U
10X ReddyMix™ 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*
DNA Template	0.5 - 10μl	0.5 - 125ng
Water (PCR Grade)	To 25μl*	

*Scale up or down the volume and concentration as appropriate

Tip:

These recommendations are intended as basic guidelines. Magnesium chloride concentration and amount of enzyme should be optimized according to template and primer combination. The gel precipitant in ReddyMix™ Buffer causes a slight increase in the thermal mass of the reaction mix. In a small number of cases this may necessitate some minor re-optimization of the thermal cycler program. If this is the case we suggest increasing the temperature of the denaturation step by 1–2°C and decreasing the temperature of the annealing step by 1–2°C. Alternatively, increase the duration of each step by 5–10 seconds.

Example of Program:

	Temp.	Time	Number of cycle
Initial Denaturation	95°C	15 min	1 cycle
Denaturation	95°C	25 sec	30 to 40 cycles
Annealing	48-63°C	35 sec	
Extension**	72°C	65 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.

Buffer composition

Enzyme Storage and Dilution	100mM	KCl
Buffer:	20mM	Tris-HCl, pH 8.0 (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-1950/A	Thermo-Start™ with 10X ReddyMix™ PCR Buffer	250 units
AB-1950/B	Thermo-Start™ with 10X ReddyMix™ PCR Buffer	2500 units

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