

## Thermo-Start™ HP ReddyMix™ 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**.

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
High Performance ReddyMix™ PCR Buffer (red)	1.25ml	10 x 1.25ml
MgCl <sub>2</sub> (clear)	1.25ml	10 x 1.25ml
dNTP Mix (brown)*	0.5ml	3 x 1.6ml

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

**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 High Performance ReddyMix™ PCR Buffer	2.5μl	1X
dNTP Mix (20mM)	1μl	0.2mM of each nucleotide
MgCl <sub>2</sub> (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
<b>Initial Denaturation</b>	<b>95°C</b>	<b>15 min</b>	<b>1 cycle</b>
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-1951/A/N	Thermo-Start™ HP ReddyMix™ PCR Kit	250 units
AB-1951/B/N	Thermo-Start™ HP ReddyMix™ PCR Kit	2500 units

All sizes are supplied with 10X Reaction Buffer, 20mM dNTP Mix and 25mM MgCl<sub>2</sub>.

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim (such as the patented 5' Nuclease Process claims in US Patents Nos. 5,210,015 and 5,487,972), no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.