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ThermoPrime *Taq* DNA Polymerase with 10X ReddyMix PCR Buffer

Description: An ultrapure recombinant thermostable DNA polymerase obtained by high level expression of the *Taq* DNA polymerase gene in *E. coli*. The enzyme exhibits enhanced thermal stability at DNA denaturation temperatures and can be shipped at ambient temperature with no loss of activity. It is licensed and optimised for use in the Polymerase Chain Reaction (PCR) process.

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: ThermoPrime has 5' to 3' polymerization and exonuclease activity but lacks 3' to 5' exonuclease activity (proofreading).

Kit Contents

Vial	Pack Size (cap color)	
	A	B
ThermoPrime	50µl (clear)	10 x 50µl (clear)
ReddyMix™ Buffer IV	1.25ml (blue)	10 x 1.25ml (blue)
$MgCl_2$	1.25ml (clear)	10 x 1.25ml (clear)

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Storage Conditions: Store thermostable DNA polymerase at -20°C. 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
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
ThermoPrime (5U/µl)	0.125µl	0.625 U
Water (PCR Grade)	To 25µl*	

*Scale up or down the volume and concentration as appropriate

Tip: 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-optimisation of the thermal cycler programme. 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	2 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.

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Buffer composition

Enzyme Storage and Dilution Buffer:	100mM	KCl
	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
ReddyMix™ Reaction Buffer (10X):	750mM	Tris-HCl, pH 8.8 (at 25°C)
	200mM	$(NH_4)_2SO_4$
	0.1% (v/v)	Tween® 20
		Red dye and precipitant

Ordering Information:

AB-0790/A	ThermoPrime with 10X ReddyMix™ PCR Buffer IV, separate vial of 25mM $MgCl_2$	250 units
AB-0790/B	ThermoPrime with 10X ReddyMix™ PCR Buffer IV, separate vial of 25mM $MgCl_2$	2,500 units

For technical information or troubleshooting contact Thermo Scientific Genomics Tech Support:

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