

Simpler Red™ DNA Polymerase

Description: Simpler Red™ DNA Polymerase contains an inert red dye to facilitate accurate low volume pipetting and as an indicator of enzyme addition. This dye has no adverse effect on the outcome of PCR. The enzyme exhibits enhanced thermal stability at DNA denaturation temperatures.

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: Simpler Red™ DNA Polymerase 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
Simpler Red DNA Polymerase	50μl (clear)	10 x 50μl (clear)
Reaction Buffer IV	1.25ml (blue)	10 x 1.25ml (blue)
MgCl ₂	1.5ml (clear)	10 x 1.5ml (clear)

<u>Simpler Red</u>	100mM	KCl
<u>DNA</u>	20mM	Tris-HCl, pH 8.0 (at 25°C)
<u>Polymerase:</u>	0.1mM	EDTA (ethylenediaminetetraacetic acid)
	1mM	DTT (dithiothreitol)
	0.5%	Tween® 20
	0.5%	Nonidet® P40
	50% (v/v)	Glycerol

<u>Reaction</u>	750mM	Tris-HCl, pH 8.8 (at 25°C)
<u>Buffer IV (10X):</u>	200mM	(NH ₄) ₂ SO ₄
	0.1% (v/v)	Tween® 20

<u>MgCl₂:</u>	25mM	MgCl ₂
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For Research Purposes Only

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Storage Conditions:

Store Simpler Red 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
Simpler Red DNA Polymerase (5U/μl)	0.125μl	0.625 U
10X Reaction Buffer IV	2.5μl	1X
dNTP Mix (20mM)	1μl	0.2mM of each nucleotide
MgCl ₂ (25mM)	1.5μl*	1.5mM*
Primer forward (10μM)	1.25μl*	0.5μM*
Primer reverse (10μM)	1.25μl*	0.5μM*
Water (PCR Grade)	Variable	
DNA Template	0.5 - 10μl	0.5 - 125ng
Total volume	25μl	

*Scale up or down the volume and concentration as appropriate
MgCl₂ concentration is usually between 1.5 and 4.0mM

Example of Program:

	Temp.	Time	Number of cycle
Initial Denaturation	94°C	2 min	1 cycle
Denaturation	94°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, Simpler Red DNA Polymerase extends at approximately 1000 bp/min.

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
	250µM	β-mercaptoethanol
	250µM	of each: dCTP, dGTP, dTTP
	1.25µg/µl	[³ H] dATP (0.05 Ci/mmol)
		activated salmon sperm DNA

Water added to a total volume of 50µl. Incubated at 74°C for 10 minutes.

Ordering Information:	AB-1605/A	Simpler Red™ DNA Polymerase	250 units
	AB-1605/B	Simpler Red™ DNA Polymerase	2500 units

All sizes are supplied with 10X Reaction Buffer IV 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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