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SMART DNA Polymerase

Code EME012500

For research use only

Cat#	Size	Description	Volume
EME012500	1x500 units	SMART DNA Polymerase	1 x 250 µl
		2.5X Reaction Buffer	6 x 2 ml

Storage and stability

Store at -18°C/-22°C for 18 months.

SMART DNA polymerase is a robust thermostable polymerase with high fidelity features. This recombinant enzyme contains a Pfu modified domain for proof reading and a DNA binding domain for high processivity. The use of SMART DNA polymerase in PCR reactions results in higher yield, shorter amplification time and amplification of long (up to 10 kb) and difficult templates (i.e. GC-reach). The enzyme has the 5'->3' DNA polymerase activity, 3'->5' exonuclease activity, temperature-dependent strand-displacement activity and generates blunt ends in the amplification products.

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 minutes at 75°C under assay conditions: 25 mM TAPS-HCl, pH 9.0 (at 25°C), 100 mM KCl, 1.5 mM $MgCl_2$, 1 mM ß-mercaptoethanol, 200 µM each dNTP, and 10 µg activated calf thymus DNA in 50 µl.

Storage & Dilution buffer

20 mM Tris-HCl, pH 8.0, 100 mM KCl, 0.1mM EDTA, 1 mM DTT, 50% Glycerol, 0.5% Tween 20 and stabilizers.

2.5X Reaction buffer

SMART DNA Polymerase is supplied with the 2.5X Reaction Buffer (proprietary formulation) containing 3.75 mM $MgCl_2$.

Application protocol

The optimal reaction conditions for SMART DNA Polymerase may differ from PCR protocols for standard (Taq-like) DNA polymerases. SMART DNA polymerase works better at high denaturation and annealing temperatures.

Note! It is critical that SMART DNA Polymerase is the last component added to the PCR mixture, since the enzyme exhibits 3'->5' exonuclease activity that can degrade primers in the absence of dNTPs.

PCR reactions can be set up at room temperature, because of very low background activity of enzyme during PCR setup conditions.

PCR reaction conditions

Reagent	Volume	Final Concentration
H_2O	23.6 ul	
2.5X Reaction Buffer	20 ul	1X
25 mM dNTPs	0.4 ul	0.2 mM
Primer FWD	0.25 ul	0.5 uM
Primer REV	0.25 ul	0.5 uM
SMART DNA Polymerase	0.5 ul	0.02 U/ul (1 U tot)
DNA	variable	<20 ng/50 µl
Final Volume	50 ul	

Reaction Components

1.SMART DNA Polymerase

An optimal amount of enzyme in 50 ul reaction is 1U. For long amplicons, the amount of SMART DNA Polymerase could be increased to 1.5 U per reaction.

2.Buffer

2.5X Reaction Buffer provides very high reproducibility across a wide range of amplification conditions, including "fast-PCR" (reduced time of PCR reaction). 2.5X Reaction Buffer contains 1.5 mM Mg2+, as final concentration. **Mg concentration should be increased up to 3.5 mM (1X) or higher when blood, stabilized by heparin/citrate or EDTA is used as DNA template.**

3.Primers

Usually 15-20 pmol of each specific primer is enough to get good PCR results. If you are using 2-step PCR with whole blood as a template, it's better to use >= 20 pmol of each primer.

CYCLING CONDITIONS

	2-step amplification		3-step amplification		
Cycle step	**T°C**	**Time**	**T°C**	**Time**	**Cycles**
Initial Denaturation[1]	98°C	2-5 min	98°C	2-5 min	1
Denaturation[2]	98°C	5-10 sec	98°C	5-10 sec	
Annealing	-	-	55-72*	10-30 sec	25-35
Extension	66-72°C	15-30 sec/kb**	72°C		
Final extension	72°C	2-5 min	72°C	2-5 min	1
	4°C	hold	4°C	hold	

* For SMART DNA Polymerase Tm of the primers should be corrected, as +3-5°C, comparing with the Tm used with Taq-based PCR.

1) Initial denaturation for 5 min at 98°C is necessary only for blood cell lysis in case of direct amplification from blood samples.
2) For most applications, 5 sec at 98°C is enough for DNA denaturation during PCR step.

NOTE: do not use denaturatin temperature lower than 98°C; it can cause troubles in PCR (non specific amplification, poor yield of PCR product, etc.).

ANNEALING/EXTENSION

For SMART DNA Polymerase annealing and extension steps should be combined in one step if:
- Tm of both primers do not differ dramatically (<3°C)
- Tm of the primers are >65°C (optimal Tm for the primers lays between 65-70°C)

If primers Tm is about 60-61°C for both primers you can apply the formula (LowerTm +72°C)/2 to determine the annealing /elongation temperature.
To determine a better annealing/elongation temperature it could be useful to run gradient amplification.
To avoid non specific band formation/smearing during amplification do not exceed extension time of 30 seconds and use the highest ramp rate of PCR system (Ramping >4-5°C is preferable).

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For non-complex DNA templates (plasmid DNA, phage DNA, BAC clone) extension time could be reduced up to 15 sec/Kb.
For complex DNA templates (human genomic DNA) if the predicted amplicons are longer than 1.5-2 Kb, extension time of 30 sec is strongly recommended.
For DNA templates with GC content less than 65% and up to 500 bp in length an extension time of 15 sec per cycle is recommended.

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EuroClone S.p.A.
Via Figino 20/22, 20016 Pero (MI) - Italy
Phone: +39.02.38195.1 - Fax +39.02.38101465
e-mail: info@euroclone.it

CELBIO BIOAIR MOLECULAR BIOLOGY

www.euroclone.it