

Description: SD Polymerase HotStart is a novel artificial thermostable Polymerase, providing a strong strand displacement activity. Unlike natural enzymes with strong strand displacement activity as Phi29 or Bst Polymerase, which are active below 68 °C only, SD Polymerase HotStart is stable up to 93 °C. This allows running isothermal amplification like LAMP with initial heating-up for increased specificity. SD Polymerase has 5'–3' Polymerase and 5'–3' strand displacement activities. It does not have any exonuclease activity. The enzyme does 3'-A-overhangs and is blocked by an antibody. SD Polymerase HotStart effectively directs PCR with templates up to 20 kb in length. A non-HotStart version of SD Polymerase is available with 10 U/μL.

Content

Ref No.	S108902	108902	108910	color
SD Polymerase HotStart	Sample size	200 units	1000 units	blue
SD Polymerase Reaction Buffer incomplete * (10x)	0.5 mL	1.8 mL	2x 1.8 mL	red
MgCl ₂ 100 mM	1 mL	1 mL	1x 1 mL	green
Datasheet	1	1	1	--

* pH 8.9, with Tween-20 and BSA; please add MgCl₂ to 3-3.5 mM end concentration just before use. Adding MgCl₂ to the 10x buffer may precipitate at 4 °C storage.

Applications: PCR with SD Polymerase is effective on long template, with extremely low concentrations of template. SD Polymerase can be effectively used in PCDR (Polymerase Chain Displacement Reaction), a combination of PCR and strand displacement.

Concentration: 10 units/μL

Sensitivity: detection of ≥ 10 DNA molecules

Unit definition: One unit of activity is defined as the amount of enzyme required to incorporate 10 nmoles of dNTP into acid-insoluble DNA fraction in 30 minutes at 68 °C.

Additionally provided: 1 tube MgCl₂ (100 mM)

Recommended MgCl₂ concentration: 3 mM – 3.5 mM

Quality Control

- 98% protein homogeneity in 10% SDS-PAGE
- No detectable exo-/endonuclease activities
- PCR and strand displacement with various templates
- Hotstart efficiency test

Storage condition: -20 °C

Important instructions

LAMP (Loop-mediated isothermal amplification): Optimum for LAMP amplification is 62 – 68 °C. High thermostability of the enzyme allows carrying out LAMP with an initial DNA denaturation step (92 °C for 2 min), which is enhancing the reaction in some applications. LAMP can be performed with 15 – 50 units of SD Polymerase per 50 µL.

PCR: SD Polymerase Hotstart is suitable for amplification of short (from 100 bp) and long (up to 20 – 30 kb) DNA fragments from simple (plasmids) and complex (genomic DNA) templates. In PCR applications SD Polymerase Hotstart demonstrates higher yields, speed and efficiency compared with *Taq*. Even single copy templates are amplified with good results.

We recommend 92 °C for denaturation steps, 68 °C for elongation steps of PCR and 0.5 – 2 units of SD Polymerase per 50 µL for PCR.

PCDR (Polymerase Chain Displacement Reaction): provides much higher efficiency and sensitivity than PCR. SD Polymerase is the enzyme of choice to run PCDR. Here the enzyme has an outstanding performance due to its unique combination of strand displacement and thermostability.

We recommend 92 °C for denaturation steps, 68 °C for elongation steps of PCDR and 0.5 – 2 units of SD Polymerase per 50 µL for PCDR.

Example for usage in PCR or PCDR: The 50 µL reaction mixture includes 1 unit of SD Polymerase, 1x SD Polymerase Reaction buffer; 3 mM MgCl₂; 0.375 mM dNTPs (each); 20 pmol of each inner primer, 10 pmol other primers (each); about 0.05 ng of cDNA library as a template.

PCR or PCDR thermocycler protocol

step	time	temperature
initial denaturation	2 minutes	92 °C
Number of cycles: 25 - 30		
denaturation	30 seconds	92 °C
annealing	30 seconds	60 °C *
extension	30 seconds	68 °C

* Usually the optimal annealing temperature is 5 °C below the melting temperature of the primers.

Notes:

Program the cycler according to the manufacturer`s instructions. Each program should start with an initial denaturation step at 92 °C for 2 min.

Recommended elongation time is 15 – 40 sec per 1 kb of target.

For maximum yield and specificity, temperatures (annealing) and cycling times should be optimized for each new template target or primer pair.

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