SD HS DNA Polymerase

User's Instruction

Description

SD HS DNA Polymerase is a novel artificial thermostable polymerase with strong strand displacement activity, but lacking 5'-3' exonuclease activity. Unlike natural enzymes with strong strand displacement activity such as Phi29 or Bst polymerase, which are active only below 68°C, SD HS DNA polymerase is stable up to 90°C. Therefore, SD HS DNA polymerase is particularly effective for PCDR, a new PCR assay that incorporates strand displacement, particularly useful in creating more sensitive qPCRs. With hot start property, the polymerase can be recovered only after heating at 90°C for 5 min.

Kit Contents

	1,000U
1. SD HS DNA Polymerase (10 U/μl)	100 μl
2. 4×SD Buffer (Mg^{2+} free)	1 ml×2
3. 100 mM Mg^{2+}	1 ml

Features

- Strong strand displacement activity and polymerase activity.
- Stable at high temperature.
- Tolerable to ethanol, guanidine salt, heparin, serum and plant polysaccharide polyphenols.
- Ideal for long and complex templates amplification.
- With hot start property, the polymerase is 100% inactive below 50°C and can be completely recovered only after heating at 90°C for 5 min.

About PCDR

Polymerase chain displacement reaction (PCDR) uses multiple nested primers in a rapid, capped, one-tube reaction that increases the sensitivity of normal quantitative PCR (qPCR) assays. In PCDR, when extension occurs from the outer primer, it displaces the extension strand produced from the inner primer by utilizing a polymerase that has strand

displacement activity. This allows a greater than 2-fold increase of amplification product for each amplification cycle and therefore increased sensitivity and speed over conventional PCR. Increased sensitivity in PCDR would be useful in nucleic acid detection for viral diagnostics.

Protocol (For PCDR)

1. Set up PCDR reaction as the following table (take 50 µl per well as an example):

Component	Volume
4×SD Buffer (Mg^{2+} free)	12.5 µl
SD HS DNA Polymerase (10 U/µl)	0.25 µl
100 mM Mg^{2+}	1.5 µl
dNTP Mixture (10 mM each)	1 µl
Template DNA	X ul
Inner Primer each (10 µM)	0.5-2 µl
Outer Primer each (10 µM)	0.5-2 µl
ddH_2O	up to 50 µl

Note:

- The amount of Mg^{2+} is usually around 2-3 mM in PCDR systems.
- The amount of SD HS DNA Polymerase is usually around 2.5 U in a 50 µl reaction system.

2. Thermocycling Conditions

Number of Cycles	Temperature	Time
1st Cycle	90°C	5min
25-35 Cycles	90°C	10s
	50-60°C	20s
	68°C	2kb/min
Last Cycle	68°C	2min

Note:

- The activity of SD HS DNA polymerase can only be restored after heating at 90°C for 5 min, thus this step cannot be shortened or omitted.

Storage

Minimum shelf life is 2 years at -20°C.