Hot-Start DNA Polymerase

Catalog Number: NP041010120 – 500 U

Description:

This DNA polymerase is a mixture of Taq DNA polymerase and a temperature sensitive, aptamer-based inhibitor. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 40 °C, but releases the enzyme during normal PCR cycling conditions. The aptamer-based hot start mechanism does not require a separate high temperature incubation step to activate the enzyme. The enzyme is inactive at room temperature, avoiding extension of non-specifically annealed primers or primer dimers and providing higher specificity of DNA amplification.

The activated enzyme maintains the same functionality as Taq DNA polymerase: it catalyzes 5' → 3' synthesis of DNA, has no detectable 3' → 5' proofreading exonuclease activity.

Contents:

Components	500U
HS Taq DNA poly. 2.5 U/µl	500U
$MgCl_2$ Solution 25 mM	1 mL
10X Buffer $MgCl_2$ free	1 mL

General Reaction Protocol:

1. Thaw 10X reaction buffer, dNTP mixture.
2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
3. Add templates DNA to the individual PCR tubes or wells containing the master mix.

Component	Volume	Final conc.
10X Reaction Buffer	2 µL	1X
$MgCl_2$ Solution 25 mM	1.2 µL	1.5 mM
40 mM dNTPs Mix (10 mM each)	0.4 µL	0.2 mM
Forward Primer (10 pmol/ µL)	1 µL	0.5 pmoles/µL
Reverse Primer (10 pmol/ µL)	1 µL	0.5 pmoles/µL
Template DNA	Variable	10 fg to 1 µg
PCR grade water	Up to 20µL final volume	-
Apta DNA poly. (2.5 units/µl)	0.25 µL	0.065 U/µl
Total Volume	**20 µL**	

4. Program the PCR machine according to the program outlined.

Cycle	Time	Temp °C
1	5 min	95
30 - 35	30 sec	94
	30 sec	57
	30-60 sec	72
1	5 min	72

Notes:

Extension temperature is between 68 and 72°C. We highly recommend 68 °C for more efficiency of Pars Tous Taq DNA polymerase.

* For PCR products longer than 3~4 Kb, use an extension time of approximately 1 min per Kb DNA.

* A DNA fragment which is amplified by Taq DNA polymerase has A overhang, and it enables you to do cloning by using T-vector.

Agarose Gel Electrophoresis:

Run the total 5-7 µL of PCR products alongside 3 µL DNA marker on a 2% agarose gel containing Green Viewer Dye DNA safe stain.