Hot-start Apta-Taq DNA polymerase

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.

Buffers and Reagents:

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.5 mM EDTA, 0.1 mM DTT, 0.5% Tween 20,0.5% Nonidet P-40, 50% Glycerol 10X *Reaction Buffer:* Contains Tris-HCl (pH 9.0), PCR enhancers, (NH4)2SO4.

Contents:

Component	Volume
Hot-Start Taq DNA poly. 2.5 U/µl	**250 U**
10 X Buffer (Mg free)	**1000 µL**
$MgCl_2$ 25mM	**1000 µL**

Kit storage:

This kit should be stored at -20°C. Unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for one year from the date of production.

General Reaction Protocol:

1. Thaw 10X reaction buffer, dNTP mixture.
2. Prepare a master mix.
3. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.

Component	Volume	Final conc.
10X Reaction Buffer	5 µL	1X
10 mM dNTPs Mix	1~5 µL	0.2~1.0 mM
Upstream Primer	Variable	0.1~1.0 µM
Downstream Primer	Variable	0.1~1.0 µM
***HS -Taq* (2.5 units/µL)**	0.2~1.0 µL	0.5~2.5 units
Template DNA	Variable	10 fg~1 µg
Sterilized D.W.	Variable	-
Total Volume	50 µL	

4. Add templates DNA to the individual PCR tubes or wells containing the master mix.

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

Cycle	Time	Temp °C
1	**5 Min**	**94**
25 ~35	**30 Sec**	**94**
	30 Sec	**50~60**
	30 ~60 Sec	**68#**
1	**5 Min**	**72**

6. Place the PCR tubes or PCR plates in the terminal cycler and start the cycling program.

7. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or any other means.

Extension temperature is between 68 and 72°C. We highly recommend 68 °C for more efficiency of Pars Tous HS-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 *HS Prime Taq* DNA polymerase has A overhang, and it enables you to do cloning by using T-vector.