KIAGENE FANAVAR

Hot-start Taq DNA polymerase

Cat. No:
FPLF003.0250
FPLF003.0500

Contents:

Component	100RXN	200RXN
Hot-start Taq DNA poly. 5 U/µl	50 µl	100 µl
MgCl2 Solution 25 mM	500 µl	1 ml
10X Buffer MgCl2 free	500 µl	1 ml

Description:

This DNA polymerase is a mixture of Taq DNA polymerase and a temperature sensitive ,aptamerbased 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 aptamerbased hot start mechanism does not require a separate high temperature incubation step toactivate 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.

Kit storage:

This kit should be stored at -20 ºC. Under this condition reagents are stable for two years from the date of production.

Protocol:

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

Component	Volume	Final Conc.
10X Reaction Buffer	2 µl	1X
MgCl2 Solution 25 mM	1.2 µl	1.5 mM
40 mM dNTPs Mix (10 mM each)	0.4 µl	0.2 mM
Upstream Primer (10 pmol/ µL)	1 µl	0.5 pmoles/µl
Downstream Primer (10 pmol/ µL)	1 µl	0.5 pmoles/µl
Template DNA	Variable	10 fg~1 µg
PCR grade water	Variable	-
Hot-start DNA poly. 5 U/µl	0.25 µl	0.065 U/µl
Total Volume	20µl	-

3) Add templates DNA to the individual PCR tubes or wells containing the master mix.
4) Program the PCR machine according to the program outlined.

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

Note:

* Extension temperature is between 68 and 72°C. We highly recommend 68 °C for more efficiency of Hot start 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 3µL DNA marker on a 2% agarose gel containing Green viewer DNA safe stain.