

## **cHot-start Tag DNA polymerase**

(250 U, 50 μl) (500 U, 100 μl)

Store at -20 °C

#### Contents:

#### Component

cHot-start Taq DNA poly	5 U/μl	250 U	500 U
MgCl <sub>2</sub> Solution 25 mM		0.5 ml	1 ml
10X Buffer MgCl <sub>2</sub> free		0.5 ml	1 ml

## **Description:**

This DNA polymerase is a chemically modified Taq DNA polymerase. The inhibitor binds reversibly to the enzyme, inhibiting polymerase activity at temperatures below 60°C, but releases the enzyme during normal PCR cycling conditions. The chemically-modified hot start mechanism requires a 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'  $\rightarrow$  3' synthesis of DNA, has no detectable 3'  $\rightarrow$  5' proofreading exonuclease activity.

## Kit storage:

This kit should be stored at -20  $^{\circ}$ C. Under this condition reagents are stable for two years from the date of production.

#### **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.

Component	Volume	Final Conc.	
10X Reaction Buffer	2 μL	1X	
MgCl2 Solution 25 mM	1.2 μL	1.5 mM	
40 mM dNTPs Mix	0 5	0.2 mM	
(10 mM each)	0.5 μL		
Upstream Primer	11	0.5	
(10 pmol/ μL)	1 μL	pmoles/μL	
Downstream Primer	1 µL	0.5	
(10 pmol/ μL)	1 μι	pmoles/μL	
Template DNA	Variable	10 fg~1 μg	
PCR grade water	Variable	-	
cHot-start Taq DNA	0.21	0.075.11/1	
poly. 5 U/μl	0.3 μL	0.075 U/μl	
Total Volume	20 μL	-	

- 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	15 min	95
	30 sec	94
30-35	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 Tag DNA polymerase.

# \* It is not suitable For PCR products longer than 0.5 Kb.

\* 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  $\mu$ L of PCR products alongside  $3\mu$ L DNA marker on a 2% agarose gel containing Green viewer DNA safe stain.

#### **Disclaimers and Addresses:**

This product is for **Research Use Only** and should only be used by trained professionals.

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