

cHot-start Tag DNA polymerase

(250 U, 100 µl) (500 U, 200 µl)

Store at -20 °C

Contents:

Component

cHot-start Taq DNA poly. 2.5 U/μl	250 U	500 U
MgCl ₂ Solution 25 mM	0.5 ml	1 ml
10X Buffer MgCl ₂ 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)	Ι μι	pmoles/μL	
Template DNA	Variable	10 fg~1 μg	
PCR grade water	Variable	-	
cHot-start Taq DNA poly.	0.3-0.4 μL	0.037-0.05	
2.5U/μl	υ.5-υ.4 μι	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 μ L of PCR products alongside 3μ 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.

Unit 18, North East Food tech park, Tous Industrial Zone, Mashhad-IRAN
Tel:(+98-51)35420843-5
Fax: (+98-51) 35420846
www.parstous.com
info@aarstous.com