Intact Genomics®

Hot Start *Taq* DNA Polymerase

Catalog #	3291	3293
Package Size	200 units	1,000 units
Volume	40 µl	200 µl
Concentration	5 units/µl	

Catalog #	3291d	3293d
Package Size	200 units	1,000 units
Volume	40 µl	200 µl
Concentration	5 units/µl	

*Catalog numbers ending with "d" include separate dNTP mix.

Description

Intact Genomics Hot start *Taq* is a thermostable DNA polymerase that possesses a 5′→3′ polymerase activity (1, 2) and a 5′ flap endonuclease activity (3, 4). Hot Start *Taq* DNA Polymerase is chemically modified that leads to complete inactivation of the polymerase until the initial heat activation step at the start of PCR. Hot start PCR reduces non-specific amplification during setup stages of the reaction and helps increase PCR specificity and sensitivity. This product is supplied with the unique Intact Genomics 10x PCR reaction buffer, containing MgCl2, which produces a final Mg2+ concentration of 1.5 mM, and 5X Magic Enhancer that enables efficient amplification of GC rich templates up to 84%.

Protein Purity

The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

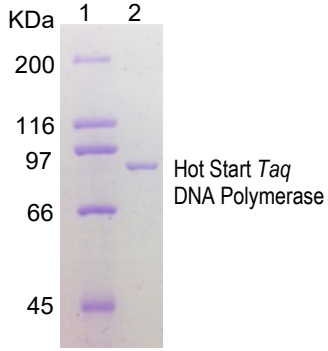


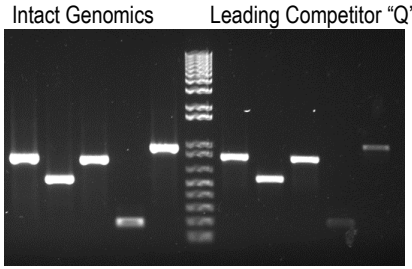
Fig. 1: Lane 1. Protein Marker
Lane 2. Hot Start *Taq* DNA Polymerase

Product Source

E. coli strain expressing a *Taq* DNA Polymerase gene from Thermus aquaticus YT-1.

Hot start *Taq* Polymerase Comparison Data:

We compare our Hot Start *Taq* Polymerase with leading competitor side by side and our enzyme is better than the competitor. A typical gel picture is shown below:



Applications

- Routine PCR
- Primer extension
- Colony PCR
- Efficient for amplifying high GC template DNA with Magic Enhancer

Product Includes

1) Hot Start *Taq* DNA Polymerase
2) 10x PCR Buffer with Mg2+
3) 5x Magic Enhancer
4) 10 mM dNTP (Cat. # 3291d, 3292d only)

Storage Temperature

–20 °C

Storage Buffer

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25 ºC

10x PCR Buffer with Mg2+

100 mM Tris-HCl pH 9.0, 15 mM $MgCl_2$, 100 mM KCl, 80 mM $(NH_4)_2SO_4$, 0.5% Igepal CA 630

Unit Definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTP into acid-insoluble form in 30 minutes at 72 ºC.

Protocol

1. Thaw 10x PCR Buffer, dNTP mix, primer solutions, 5X Magic Enhancer (if required) and mix thoroughly before use.
2. Prepare a reaction mix according to the following table: The reaction mix typically contains all the components needed for PCR except the template DNA.

PCR Reaction Set Up:	
Template DNA	x µl (0.01-0.5 µg)
10x PCR Buffer	10.0 µl
dNTP (10 mM)	2.0 µl
Forward Primer	x µl (0.1- 0.5 µM)
Reverse Primer	x µl (0.1- 0.5 µM)
5x Magic Enhancer (optional)	20 µl
Hot start *Taq* DNA Polymerase (5 U)	0.5 µl
H_2O up to	100.0 µl

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3. Mix the reaction mixture thoroughly.
4. Add template DNA to the individual PCR tubes containing the reaction mixture.
5. Program the thermal cycler according to the manufacturer's instructions.

Note: PCR program must start with an initial heat-activation step at 95 °C for 15 min.

A typical PCR cycling program is outlined in the following table.

PCR Cycling Conditions:			
Steps	**Temp.**	**Time**	**Cycles**
Initial denaturation	95 ºC	15 min	1
Denaturation	94 ºC	30-60 sec	25-35
Annealing	52-66 ºC	30-60 sec	
Extension	72 ºC	1-2 min	
Final extension	72 ºC	10 min	1
Hold	4-12 ºC	∞	

6. Place the PCR tubes in the thermal cycler and start the cycling program.

Reference

1. EChien, A., Edgar, D.B. and Trela, J.M. (1976). J. Bact. 127, 1550-1557.
2. Lawyer, F.C. et al. (1993). PCR Methods and Appl. 2, 275-287.
3. Longley, M.J., Bennett, S.E. and Mosbaugh D.W. (1990). Nucleic Acids Res. 18, 7317-7322.
4. Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993). Science. 260, 778-783.