Hot Start i7 High-Fidelity DNA Polymerase

Catalog #	3281	3283
Package Size	200 Units	500 Units
Concentration	2 units/µl	

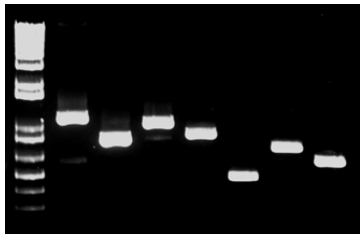
Description

Intact Genomics (IG) hot start i7 high-fidelity DNA polymerase is a genetically engineered, heat stable DNA polymerase which has 5′→3′ polymerase and 3′→5′ exonuclease (proofreading) activities. Hot Start i7 high fidelity 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 enzyme has the high-fidelity, sensitivity and processivity with an error rate ~2.8x10^2-fold lower than *Taq* DNA polymerase, and significantly lower than the error rates of other proofreading enzymes in the marketplace (1). Hot start i7 high-fidelity DNA polymerase is ideal for cloning and can be used for long (up to 20kb) or difficult amplicons. This product is supplied with the Intact Genomics proprietary 5x PCR reaction buffer containing $MgCl_2$ with a final (1x) concentration of 2 mM, and 5x magic enhancer that enables efficient amplification of GC rich templates up to 84%.

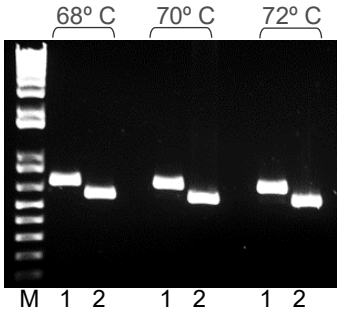
Activity data

Hot start i7 high-fidelity DNA Polymerase generates robust and high-quality PCR products with difficult templates (**Fig. A**). PCR extension temperatures can be used between 68 to 72º C (**Fig. B**). This enzyme is resistant to different PCR inhibitors such as heparin, humic acid and xylan (**Fig. C**).

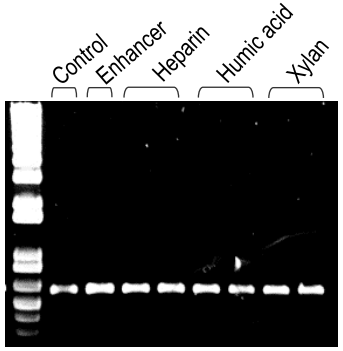
A). PCR with difficult templates



B). Different PCR extension temperatures



C). Resistant to different PCR inhibitors



Applications

- Long and difficult template DNA amplification
- Cloning
- High-fidelity PCR
- Efficient for amplifying high GC content template DNA with magic enhancer

Product Includes

- Hot start i7 High-Fidelity DNA Polymerase
- 5x i7 PCR Buffer with Mg2+
- 5x Magic Enhancer

Storage Buffer

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

Storage Temperature

-20ºC

Heat Inactivation

No

Quality Control Assays

Hot Start i7 High-Fidelity DNA Polymerase is free from detectable nuclease activities.

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 72º C.

Protocol

1. Thaw i7 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
5x i7 PCR Buffer	10.0 µl
dNTP (10 mM)	1.0 µl
Forward Primer (10 µM)	2.5 µl
Reverse Primer (10 µM)	2.5 µl
5x Magic Enhancer (optional)	(10.0 µl)
Hot start i7 High-Fidelity DNA Polymerase (2 U/µl)	0.5 µl
H_2O up to	50.0 µl

3. Mix the reaction mixture thoroughly.
4. Add template DNA to the individual PCR tube containing the reaction mixture.
5. Program the thermal cycler according to the manufacturer's instructions. A typical PCR cycling program is outlined in the following table.

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PCR Cycling Conditions:			
Steps	**Temp.**	**Time**	**Cycles**
Initial denaturation	98 ºC	10-15 min	1
Denaturation	98 ºC	10-20 sec	25-35
Annealing	54-66 ºC	10-30 sec	
Extension	68-72 ºC	10-30 sec/kb	
Final extension	68-72 ºC	5 min	1
Hold	4-12 ºC	∞	

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

References

1. Frey, B. and Suppman, B. (1995). *BioChemica*. 2, 34-35.