i7® Hot Start High-Fidelity DNA Polymerase

Intact Genomics®

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

Description

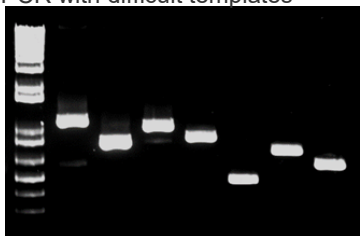
Intact Genomics (ig®) i7® Hot Start High-Fidelity DNA Polymerase is a genetically engineered, heat stable DNA polymerase which has 5´→3´ polymerase and 3´→5´ exonuclease (proofreading) activities. Hot Start i7 High-Fidelity DNA Polymerase is chemically modified that leads to complete inactivation of 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). i7® Hot Start 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 2.5x PCR reaction buffer containing $MgCl_2$ with a final (1x) concentration of 2 mM.

This buffer allows for amplification of non GC rich templates and of GC rich templates up to 84%.

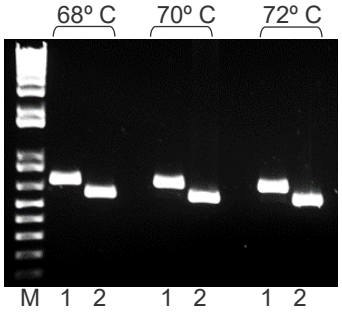
Activity Data

i7® Hot Start 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**).

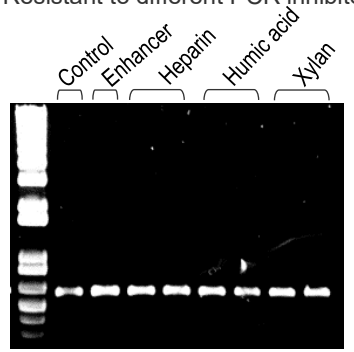
A). PCR with difficult templates



B). Different PCR extension temperatures



C). Resistant to different PCR inhibitors



Applications

- Long and difficult template DNA amplification
- Cloning
- High-fidelity PCR

Product Includes

- i7® Hot Start High-Fidelity DNA Polymerase
- 2.5x i7® PCR Buffer with Mg2+

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

i7® Hot Start 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 72ºC.

Protocol

1. Thaw i7® PCR Buffer, dNTP mix and primer solutions 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
2.5x i7® PCR Buffer	20.0 µl
dNTP (10 mM)	1.0 µl
Forward Primer (10 µM)	2.5 µl
Reverse Primer (10 µM)	2.5 µl
i7® Hot Start 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:

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.

Related Products

- Taq DNA Polymerase 2x Premix (Cat.# 3249)
- ig® 10B Electrocompetent Cells (Cat.# 1212-12)
- ig® 10B Chemically Competent Cells (Cat.# 1011-12)
- ig-Fusion™ Cloning Kit (Cat.# 4111)
- ig® Hot Start High-Fidelity DNA Polymerase (Cat.# 3281)
- i7® Hot Start High-Fidelity DNA Polymerase 2x Master Mix (Cat.# 3284)
- i7® High-Fidelity DNA Polymerase (Cat.# 3254)

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.