i7 High-Fidelity DNA Polymerase

Catalog #	3254	3255
Package Size	200 Units	500 Units
Concentration	2 units/µl	

Description

Intact Genomics (IG) i7 High-Fidelity DNA Polymerase is a genetically engineered, heat stable DNA polymerase which has 5′→3′ polymerase and 3′→5′ exonuclease (proofreading) activities. 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 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 i7 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%.

Protein Purity

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

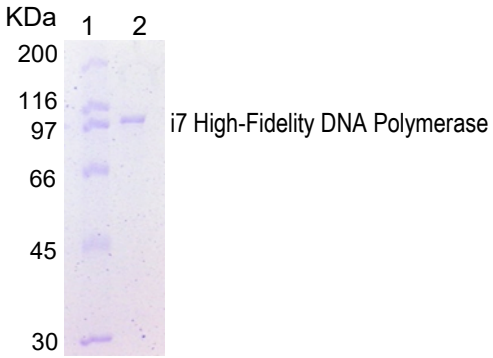


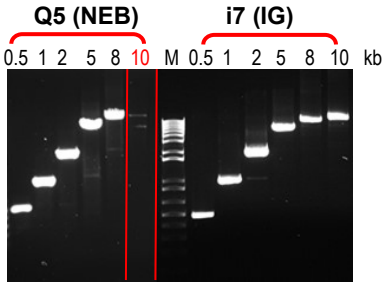
Figure 1: Lane 1. Protein marker
Lane 2. i7 High-Fidelity DNA Polymerase

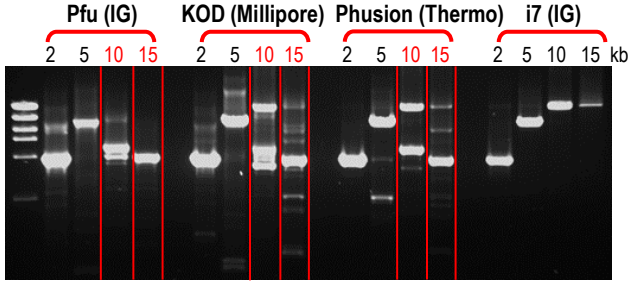
Product Source

E. coli strain expressing genetically engineered i7 High-Fidelity DNA Polymerase gene.

Comparison Data

We have tested i7 High-Fidelity DNA Polymerase activity with λ DNA and other difficult templates for PCR amplification up to 20kb. Intact Genomics (IG) i7 high-fidelity DNA Polymerase generates robust and high-quality PCR products in comparison with other high-fidelity DNA polymerases available in the marketplace (data shown below):





Applications

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

Product Includes

- 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

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 5x 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 (0.01-0.5 µg)
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)
i7 High-Fidelity DNA Polymerase (2 U/µl)	0.5 µl
H_2O up to	50.0 µl

3. Mix the reaction mixture thoroughly.

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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	1-2 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.