Hot Start i7 High-Fidelity DNA Polymerase 2x Master Mix

Catalog #	3284	3286
Package Size	100 reactions	500 reactions

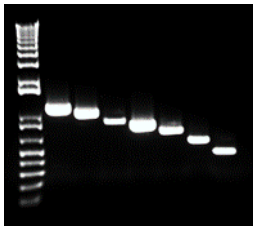
Description

Intact Genomics (IG) hot start i7 high-fidelity DNA polymerase 2x master mix is ready to use premix which contains hot start i7 high-fidelity DNA polymerase, dNTPs, $MgCl_2$, PCR enhancers and stabilizers with optimized proprietary reaction buffer. Hot start i7 high-fidelity DNA polymerase is a chemically engineered, heat stable DNA polymerase which has 5′→3′ polymerase and 3′→5′ exonuclease (proofreading) activities. This enzyme has high-fidelity, sensitivity and processivity with an error rate ~$2.8x10^2$-fold lower than *Taq* DNA polymerase, and significantly lower than other proofreading enzymes in the marketplace (1). This 2x master mix product is supplied with 5x magic enhancer that enables efficient amplification of GC rich templates up to 84%.

Activity Data

We have tested hot start i7 high-fidelity 2x master mix with difficult templates for PCR amplification. Typical PCR results are shown below:

Hot start PCR with difficult templates



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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 2x master mix
- 5x magic enhancer

Storage Temperature

-20ºC

Heat Inactivation

No

Storage Buffer

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

Protocol

1. Thaw hot start i7 high-fidelity 2x master 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).*

Components	20 µl reaction	50 µl reaction	Final concentration
Template DNA	variable	variable	1-1000 ng
Forward primer (10 µM)	1.0 µl	2.5 µl	0.5 µM
Reverse primer (10 µM)	1.0 µl	2.5 µl	0.5 µM
5x magic enhancer (optional)	(4.0 µl)	(10.0 µl)	(1x)
Hot start i7 high-fidelity 2x master mix	10.0 µl	25.0 µl	1x
H_2O up to	20.0 µl	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	52-66 ºC	10-30 sec	
Extension	68-72 ºC	10-30 sec/kb	
Final extension	68-72 ºC	5 min	1
Hold	4-12 ºC	∞	

References

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

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