

Catalog #	Package Size	Volume
3295	100 reaction (50 µl rxn vol)	2.5 ml
3296	100 reaction (50 µl rxn vol)	12.5 ml
3297	100 reaction (20 µl rxn vol)	1 ml
3299	100 reaction (20 µl rxn vol)	5 ml

Description

Hot start *Taq* DNA Polymerase 2x master mix is ready to use premix which contains hot start *Taq* DNA Polymerase, dNTPs, MgCl₂ and stabilizers with optimized reaction buffer. It has been optimized for routine PCR applications. 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 5X Magic Enhancer that enables efficient amplification of GC rich templates up to 84%.

Applications

- Routine PCR and RT-PCR
- Primer extension
- Colony PCR
- Genotyping
- Efficient for amplifying high GC content template DNA with Magic Enhancer.

Product Includes

- 1) Hot start *Taq* 2x master mix
- 2) 5X Magic Enhancer

1x Master Mix Composition

10 mM Tris-HCl pH 9.0
 50 mM KCl
 1.5 mM MgCl₂
 0.2 mM dNTPs
 5% Glycerol
 0.08% Igepal CA 630
 0.05% Tween-20
 100 Units/ml Hot start *Taq* Polymerase.

Storage Temperature

-20 °C

Protocol

1. Prepare a reaction mix according to the following table:

PCR reaction set up:	
Template DNA	1-50 ng
Forward primer (5 µM)	1.0 µl
Reverse primer (5 µM)	1.0 µl
Hot start <i>Taq</i> 2x master mix	10.0 µl
5X Magic Enhancer (optional)	4.0 µl
H ₂ O up to	20.0 µl

2. Mix the reaction mixture thoroughly.
3. Program the thermal cycler according to the manufacturer's instructions.

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

PCR cycling conditions:			
Steps	Temperature	Time	Cycles
Initial denaturation	95°C	15 min	1
Denaturation	95°C	30 sec	25-40
Annealing	50-66°C	30 sec	
Extension	72°C	1 min/kb	
Final extension	72°C	5-10 min	1

5. Place the PCR tubes in the thermal cycler and start the cycling program.
6. Analyze 5 µl of PCR products by agarose gel electrophoresis.

References:

1. Chien, 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.
2. Longley, M.J., Bennett, S.E and Mosbaugh D.W. (1990) *Nucleic Acids Res.*18, 7317-7322.
8. Lyamichev, V., Brow, M.A. and Dahlberg, J.E. (1993). *Science.* 260, 778-783.