Data sheet

2X HotBegan™ Red-Taq Master Mix

Cat. No: P0320 **Cat. No: P0321**

Introduction

2X HotBegan™ Red-Taq Master Mix is an optimized ready-to-use solution containing HotBegan Taq DNA Polymerase (hot start performance), dNTPs, $MgCl_2$ and stabilizers. It is inactive at room temperature and only requires addition of template, primers, and water.

HotBegan Taq DNA polymerase is a **Taq DNA polymerase** bound to a proprietary antibody that blocks polymerase activity until denaturation step occurs. The heat labile antibodies are rapidly inactivated by raising the temperature (4 minutes at 95-97°C). This prevents or minimizes primer-dimer and nonspecific products.

The mix also contains an agarose loading buffer including a **red dye for visual tracking** of DNA migration and a dense compound to facilitate the drop-down of the samples into the well agarose gels.

Like the Taq polymerase, the enzyme has 5'→3' polymerase activity and a weak 5'→3' exonuclease activity but no 3'→5' exonuclease activity (proofreading). Before enzyme activation none of enzyme activities are detectable.

Features

- Inactive at room temperature.
- Adds extra nucleotides (preferentially adenine) without template at 3´ends leaving 3´overhangs PCR fragments. This fact allows the popular TA-cloning or GC cloning.
- Both save times in the PCR process and in agarose loading samples.
- Amplifies from a femtograms of DNA targets.

Applications

- PCR fragments amplification for TA or GC cloning
- Design for high throughput applications.
- Amplification from a limited DNA template or low copy number genes.

Components	P0320	P0321
2X HotBegan Red-Taq Master Mix*	5x 1.25 mL	10 x 1.25 mL
50mM $MgCl_2$ Solution**	1.5 mL	1.5 mL

*2X HotBegan Red-Taq Master Mix include HotBegan Taq DNA polymerase, 2X Red buffer, 0.4 mM of each dNTP, 5 mM $Mg2^+$ and 5% Glycerol.

**Separate tube 50 mM $MgCl_2$ solution is provided for further optimisation. In some cases, we recommend to optimize $Mg2^+$ concentration.

Assay conditions

25mM Tris-HCl pH9.0 at 25°C, 50mM KCl, 2mM $MgCl_2$, 0.1mg/mL gelatine, 200 µM of dATP, dGTP, dTTP, 100µM[α32-P]dCTP (0.05µCi/nmol) and 12.5 µg activated salmon sperm DNA.

Unit definition: One unit is defined as the amount of enzyme required to catalyse the incorporation of 10 nanomoles of dNTPs into acid-insoluble material in 30 minutes at 74°C.

Quality Certifications

- Functionally tested in PCR.
- Not detectable activity of nucleases (endo-, exo, and ribo-).

Storage: Upon receipt, store the entire kit at -20 °C

(Continued on reverse side)

Recomended PCR assay

	20 µl assay	50 µl assay
2X HotBegan Red- Taq Master Mix	10 µl (1X)	25 µl (1X)
Forward Primer (15µM)	0.75 pmol/µL	0.75 pmol/µL
Reverse Primer (15µM)	0.75 pmol/µL	0.75 pmol/µL
Template DNA	plasmid: 30-75ng; gDNA: 100-500ng	plasmid: 30-75ng; gDNA: 100-500ng
Nuclease-free water	up to 20 µL	up to 50 µL

Cycling instructions:

1x 94ºC 10:00; 30x **(94ºC 0:35, Tm 0:35, 72ºC 1'/kb)**;1x 72ºC 7:00; 1x 4ºC ∞

This procedure is intended for use as a guide only and may need optimization. The optimal conditions will vary from reaction to reaction and are dependent on the template/primers used.

*Red dye Agarose Mobility**

Agarose Gel Concentration (%)	Effective separation of: (bp)	Migration Rate (bp)
0,7	800-12000	3000
1,0	400-8000	1500
1,5	200-3000	900
2,0	100-2000	300
3,0	25-1000	> 100

** in TAE Buffer*

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.