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DATA SHEET

Version: 3
Revision date: 09/04/2024

1. Identification

Product name **HotBegan™ Hot Start Red DNA Polymerase MasterMix 2X**
250 rxn

Cat. No **P0320**

2. Description

HotBegan™ Hot Start Red DNA Polymerase MasterMix 2X is an optimized ready-to-use solution containing HotBegan Taq DNA Polymerase (hot start performance), dNTPs, MgCl2 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.

3. Composition

Item	Quantity
2X HotBegan Red DNA Polymerase Master Mix*	5 x 1.25 mL
50mM MgCl2 Solution**	1.5 mL

Notes:

*2X HotBegan Red DNA Polymerase Master Mix includes HotBegan Taq DNA polymerase, 2X Red buffer, 0.4 mM of each dNTP, 4 mM Mg^{2+} and 24% Glycerol.

**Separate tube 50 mM MgCl2 solution is provided for further optimization. In some cases, we recommend to optimize Mg^{2+} concentration.

4. 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.

5. Storage specifications

Upon receipt, store the entire kit at -20 ˚C.

6. 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.

7. 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.

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8. 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.

9. Quality certifications

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

10. Further information

Product Use Limitation This product is developed, designed and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Disclaimer The information provided in this Data Sheet is correct to the best of our knowledge and belief at the date of publication. This information is intended only as a guide and should not be taken as a warranty or quality specification. Canvax Reagents S.L.U. shall not be held liable for any damage resulting from handeling or from contact with the above product.

RECOMMENDED PCR ASSAY

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

Cycling instructions:

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

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