# x-VITA™ Hot Start Taq Polymerase Master Mix

TAQM-H01-001

## Description

x-VITA Hot Start Master Mix is an optimized ready-to-use solution containing Hot Start Taq Polymerase, dNTPs, $MgCl_2$ and stabilizers. It is inactive at room temperature and only requires addition of template, primers, and water. x-VITA Hot Start Taq polymerase is 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. 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
- ✓ Amplifies from a femtograms of DNA targets

## Applications

- ✓ Real time PCR.
- ✓ RT-PCR and quantitative RT-PCR
- ✓ Genotyping with Taqman probes
- ✓ PCR fragments amplification for TA or GC cloning
- ✓ Amplification from a limited DNA template or low copy number genes

## Contains

2 x 1.25 mL x-VITA Hot Start Master Mix 2X, which includes x-VITA Hot Start Taq polymerase (0.1U/µl), 2X PCR buffer, 0.4 mM of each dNTP, 4 mM $Mg^{2+}$, 4% Glycerol.

2 x 1.25 mL Nuclease-free water.

## Assay conditions

25mM Tris-HCl pH9.0 at 25°C, 50mM KCl, 2mM $MgCl_2$, 0.1mg/mL gelatine, 200 µM de 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
- ✓ Undetected bacterial DNA (by PCR)
- ✓ Undetectable nucleases activity (endo-, exo, and ribo-)

## Storage

Store at -20°C.

## Product use limitation

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

## Recommended PCR assay (20 µl assay)

The following protocol can be used as a starting point for reaction optimization. The optimal conditions will vary from reaction to reaction and are dependent on the template/primers used.

| | |
|---|---|
| x-VITA Hot Start 2X Master Mix | 10µl (1X) |
| Forward Primer (15µM) | 1µl (0.75 pmol/µL) |
| Reverse Primer (15µM) | 1µl (0.75 pmol/µL) |
| Template DNA | Plasmid: 30-75ng<br>gDNA: 100-500ng |
| PCR grade $H_2O$ | up to 20 µl |

Cycling instructions: 94ºC 5:00, 25-30x (94ºC 0:35, Tm 0:35, 72ºC 1'/kb), 72ºC 7:00, 4ºC ∞)