



## Bio-Star one.step RT-PCR Master „EXTREME” (2X)

Productcode: 105-570

*The superior PCR Master mix kit for efficient, specific cDNA synthesis and amplification in a single tube for real-time RT-PCR even in the presence of common reaction inhibitors*

### Features:

- High specificity and High sensitivity
- Easy and convenient in use developed for RNA and DNA virus amplicons with the same plate
- Unique RNAScribe enzyme Reverse Transcriptase genetically engineered (M-MULV)
- Stable at room temperature for up to 5 days
- Reverse transcriptase can work at high temperatures (up to 60 °C)
- Standardized conditions of the same-type reactions
- PCR products can be further subjected to TA cloning due to deoxyadenosine overhangs at the ends of amplified DNA fragments.

### Description:

RNAScribe RT Reverse Transcriptase (RT) is a genetically engineered MMLV (Moloney Murine Leukemia Virus) based Reverse Transcriptase. This is an RNA-directed DNA polymerase that can synthesize a complementary DNA strand from ssRNA or ssDNA and is active over a broad range of reaction temperatures from 37°C-60°C. RNAScribe RT is a robust enzyme for RNA detection and has enhanced stability at room temperature with no activity loss for up to 1 month. This RT contains a functional RNase H domain which can increase the sensitivity of RT-qPCR (quantitative reverse transcription PCR).

### Applications:

- Gene expression analysis;
- For labs to test for both RNA and DNA viruses in a variety of samples
- One-step real-time RT-qPCR.

### Limit of use:

max. Target length: 8 kb

### Kit Components for RT-qPCR:

#### 1. BioStar Enzyme-mix (25x)

50 mM Tris-HCl (pH 8.0 at 25 °C), 100 mM NaCl, 1 mM EDTA, 5 mM DTT, 50 % (v/v) glycerol and 0.1 % (v/v) NP-40, RNAScribe RT reverse transcriptase, highly-processive HS-Taq DNA polymerase and inhibitor of RNases.

#### 2.) Reaction Buffer (2x):

100 mM Tris-HCl (pH 8.3 at 25 °C), 150 mM KCl, 0.6 mM each dNTP, 8 mM MgCl<sub>2</sub>, 10mM TCEP, stabilizer

### Storage:

@ -20°C, short term at room temperature up to 6 days.

@ +4°C for about 10 days.

About 30 times Multiple thawing/freezing without any loss of activity.

### Transport:

the product will be shipped with "blue ice"; up to 6 days @ room temperature.



## Protocols / Manuals / 25 µl total mix

- Defrost and mix all components and spin gently
- Add the volumes from the table to thin- wall PCR tubes (preferred on ice)

Component	Volume	final. conc
2x Re-action mix	12,5 µl	
25x Bio-Star Mastermix	1,0 µl *	
Forward primer	variable	0,1 - 500 nM
Reverse Primer	variable	0,1 - 500 nM
Probe	variable	0,1 - 500 nM
RNA template	variable	1 pg - 1 µg
Sterile water	up to 25 µl	

\* range from 1-3 µl

- Centrifuge your reaction chambers

## Start your PCR-Cycler routine:

Step	Temp. °C	Time	cycles
Reverse Transcription	45-55	10-15 min	1
1. Denaturation	94	3-5 min	1
Denaturation	93-94	5-15 sec	25-45
Annealing	50-68 (T <sub>m</sub> -5)	5-20 sec	25-45
Elongation	72	0,5 min/kbp	25-45

## Optimization of reaction conditions

- The reaction volume can be varied in the range of 10 to 50 µl with proportional change in the amount of all components.
- When using a template containing GC-rich regions and regions with complicated spatial structure, the temperature can be increased to 60 °C, and/or reagents facilitating melting of the secondary structure of the nucleic acids (e.g. DMSO) can be added.
- Storage terms: in a place protected from light at room temperature - 20 days at +4 °C – 2 month; at -20 °C – 1,5 years; not more than 30 thawing-freezing cycles.

## Ordering information:

Product code	Description	Amount
105-570	Bio-Star one.step RT-PCR Master Mix „EXTREME”	2 x 1,25 ml