



Bio-Star one.step RT-PCR Master Mix with SybrGreen and blue inert Dye (2X)

Productcode: 105-550

The superior PCR Master mix kit includes all the necessary components (except for DNA template and primers) for high sensitivity (up to 1 pg) and specificity amplification. The kit is designed for one-step reverse transcription or real-time polymerase chain reaction (qRT-PCR)

Features/Description:

The one-step-kit contains all reagents required for RT-qPCR in a bundle to ensure fast and easy preparation with a minimum of pipetting steps. Just add template, primers.

- The mix is coloured for easy pipetting
- High specificity
- High sensitivity (1pg – 1 µg of RNA)
- Contains an inhibitor of RNases
- error-correcting activity because of Pfu DNA Polymerase
- recommended for GC-rich templates
- templates up to 5 kb

Limited use: The use of fluorescent probes is not recommended because of blue fluorescent dye

Description:

Bio-Star Master-mix contains the optimal ratio of M-MuLV–RH to HS-Taq DNA polymerase for both reactions.

M-MuLV–RH is a genetically modified Moloney Murine Leukemia Virus reverse transcriptase (M-MuLV). The enzyme has RNA- and DNA-dependent polymerase activity but lacks RNase H activity.

M-MuLV–RH reverse transcriptase exhibits improved thermal stability and is active at high temperatures (up to 50°C).

HS-Taq DNA polymerase is a recombinant Taq DNA polymerase inactivated by specific monoclonal antibodies.

The enzyme is inactive at temperatures up to 70 °C, it is activated at the first PCR cycle during a short 5-min incubation at 95 °C. Recombinant HS-Taq DNA polymerase catalyses 5'→3' synthesis of DNA and possesses 5'→3' exonuclease activity of the native Taq DNA polymerase from *Thermus aquaticus*.

Recombinant HS-Taq DNA polymerase is ideal for conventional PCR of the templates of up to 5 kbp.

Components RT-qPCR:



1. BioStar Enzyme-mix:

M-MuLV–RH reverse transcriptase, mixture of HS-Taq DNA Polymerase (inhibited with mAB), Pfu DNA Polymerase and inhibitor of RNases, stabilizer and enhancer

2.) Re-Action buffer (2X):

100 mM Tris-HCl (pH 8.3 at 25 °C), 150 mM KCl, 0.6 mM each deoxynucleoside triphosphate, 6 mM MgCl₂, 8 mM DTT, fluorescent dye SYBR Green I and inert dye, stabilizer

3.) DEPC-treated water

4.) DMSO 0,5 ml

Note:

The absorption maximum of the inert dye is 615 nm.

Storage:

@ -20°C, short term at room temperature up to 2-3 days.

@ +4°C for about one week.

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

Transport:

the product will be shipped with "blue ice"; up to 2-3 days @ room temperature.

Protocols:

- Defrost and mix gently the 2x Re-action buffer
- 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	
Bio-Star Mastermix	1,0 µl	
Forward primer	variable	0,1 - 500 nM
Reverse Primer	variable	0,1 - 500 nM
RNA template	variable	1 pg - 1 µg
Sterile water	up to 25 µl	

Note:



- 1.) In case of amplification of templates with complicated spatial structure, DMSO can be added in the amount of 1 to 5% of the final volume of the reaction solution. Change in T_m of the primers should be taken into account when selecting the amplification program.
- 2.) Bio-Star qPCR Mastermix can volume can be in a range from 1-3 μ l.

Cycling:

- Centrifuge your reaction chambers
- Start your PCR-Cycler Program following the table below:

Step	Temp. °C	Time	cycles
Reverse Transcription	45	10 min	1
1. Denaturation	92-94	3-5 min	1
Denaturation	92-94	5-15 sec	25-45
Annealing	50-68 (T_m-5)	5-20 sec	25-45
Elongation	68	0,5 min/kbp	25-45
Melting curve	65-95		1

Ordering information:

Product code	Description	Amount
105-550	Bio-Star one.step RT-PCR Master Mix with SybrGreen and blue inert Dye	2 x 1,25 ml