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BioMaster RNAscribe RT Plus (5×)

Cat. Number: R02-100, R02-400

Description:

BioMaster RNAscribe RT Plus (5×) kit designed for the first cDNA chain synthesis in the conduct of two-step quantitative RT-PCR. The kit contains ready-to-use *BioMaster RNAscribe RT Plus (5×)* mixture, containing: thermostable revertase *RNAscribe* and RNase inhibitor for RNA-matrix protection, also with all of the necessary components for the reverse transcription reaction.

Maximum representation of the all RNA sequences as cDNA provided by the presence of random hexaprimer and oligo(dT)₁₆ primers with optimal ratio. Besides, the reagent contains inert blue dye for the visual control of reaction performance. The volume of the bas material for the reaction conduction may vary from RNA single copies up to 1 µg without quality loss of the reverse transcription.

BioMaster RNAscribe RT Plus (5×) can be used for cDNA synthesis at a temperature range of 42 – 65 °C. For the most of applications the optimal temperature is 55 °C. The kit format allows to significantly reduce the time on the first step of the two-step RT-PCR.

The kit contains *Control-RT (5×)*, being a mixture identical to *BioMaster RNAscribe RT Plus (5×)*, EXCEPT the enzyme.

Kit contains:

Component	Cat. № (Amount of 20 µl reactions)	
	R02-100	R02-400
BioMaster RNAscribe RT Plus (5×)	1 × 400 µl	2 × 800 µl
Control-RT (5×)	1 × 40 µl	1 × 160 µl
DEPC treated water	1 × 1,8 ml	4 × 1,8 ml

Contains of BioMaster RNAscribe RT Plus (5×):

100 mM Tris-HCl, pH 8.3 (at 25 °C), 250 mM KCl, 2,5 mM of each deoxynucleosidetriphosphate, 20 mM MgCl₂, 2,5 mM TCEP, enzyme stabilizers and enhancers, reverse transcriptase *RNAscribe RT*, RNases inhibitor, inert dye, random hexaprimer and oligo(dT)₁₆ primer.

Application

- First cDNA chain synthesis for RT-PCR and RT-PCR in real time;
- cDNA synthesis for cloning;
- cDNA synthesis for complicated and long matrixes;
- production of labeled cDNA probes for microarray.

Properties of the reaction mixture

- Effective performance in a wide temperature range (42 – 65 °C);
- Storage stability (up to one month at + 4 °C);
- Effective coverage (non-specific primers optimal ratio).

Application advantages

- The mixture is stained for distribution facilitation while pipetting;
- High sensitivity (10 pg – 1 µg RNA);
- Simplicity and convenience of application;
- Low pipetting error;
- Allows to standardize the setting conditions for the identical reactions (error reduction when mixing PCR components in different experiments);

Assay

Before the start of the work it is recommended to read the rules and guidelines, listed in the kit description at <http://biolabmix.ru>

General recommendation

BioMaster RNAscribe RT Plus (5×) can synthesize cDNA at the range of temperatures being 42 – 65 °C. For the most of application cases the optimal temperature is 55 °C.

We recommend to use 1 µl of reaction mixture as a matrix for the subsequent PCR. If necessary, undiluted cDNA can compose up to 25% of the PCR reaction mixture volume. For the work convenience, the obtained after the reaction cDNA can be diluted 5 times (up to 100 µl) with nuclease-free water and put 5 µl in the subsequent PCR.

Reverse transcription (first cDNA chain synthesis)

1. Carefully vortex mixture *BioMaster RNAscribe RT Plus (5×)* and discard the droplets.
2. Prepare the reaction mixture to obtain the first cDNA chain as described below:

Component	For one reaction (20 µl)	Final concentration
BioMaster RNAscribe RT Plus (5×)	4 µl	1×
RNA sample	variable	Up to 1 µg
DEPC treated water	Up to 20 µl	

3. Incubate in the amplification according to the next assay:

Stage	Temperature, °C	Time, min
Primers annealing	25	2
cDNA synthesis	55	10*
Inactivation	95	1

*indicated time is for the short fragments with the subsequent real-time PCR analyses. If the longer fragments are planned to be analyzed, it is recommended to increase time up to 30-50 min.

Storage: at +4 °C – 1 month, at -20°C – 1 year; max. of 30 freeze-thaw cycles.

Transportation: in thermocontainers with cooling elements; the temperature may increase up to the ambient temperature if transportation lasts less than 7 days.