PRODUCT	SIZE	SKU
VitaScript™ FirstStrand cDNA Synthesis Kit	50 rxn / 20 µl	TRAXSKU1301

DESCRIPTION

VitaScript™ First Strand cDNA Synthesis Kit contains:

COMPONENT	VOLUME	DESCRIPTION
VitaScript™ Enzyme Mix	50 µl	contains VitaScript™ Reverse Transcriptase and RNase inhibitor blend
5X VS Reaction Buffer	250 µl	contains dNTPs, $MgCl_2$, random 6-mer primers and oligo-dTs in an optimized buffer environment
Nuclease-free water	1 ml	

GENERAL CONSIDERATIONS

- High purity template RNA is essential for reliable efficient cDNA synthesis. A A_{260} / A_{280} ratio of 1.7 or higher is strongly recommended.
- The amount of template RNA is depended on the expected copy number of the sequence of interest. In general, 1 µg – 1 ng of total RNA is recommended, 0.05 – 100 ng if you are working with isolated mRNA.
- When working with long cDNA synthesis, denaturation of RNA with VS reaction buffer for 5 minutes at 72°C can be applied to remove secondary structures that can impede the reaction.
- This protocol recommends cDNA synthesis for 1 hour at 42°C.
- To enhance the template coverage, the VS reaction buffer also contains random hexamer primers. This provides multiple priming sites along the RNA for the detection of multiple short sequences.

REACTION SETUP

Thaw all components and mix gently. Keep on ice during reaction setup. A control reaction without VitaScript™ Reverse Transcriptase is highly recommended to check for potential DNA contamination.

(*Optional*) Denature RNA with VS Reaction Buffer for 5 minutes at 72°C. Spin down and instantly put on ice. This can improve transcription for long mRNAs or GC-rich RNA.

- As negative control, replace VitaScript™ Enzyme Mix with 1 µl nuclease-free dH_2O.
- Mix in a sterile RNase-free tube:

COMPONENT	VOLUME
5X VS Reaction Buffer	4 µl
VitaScript™ Enzyme Mix	1 µl
Total RNA	1-6 µl
Nuclease-free dH_2O	to 20 µl
total volume	20 µl

STEP	TEMPERATURE	TIME
cDNA Synthesis	42°C	60 minutes
Inactivation of VitaScript™	80°C	10 minutes

- Dilute the reaction with nuclease-free dH_2O to 200 µl and store at -20°C. Avoid repeated freeze-thaw cycles.

For following PCR applications, the diluted cDNA reaction should represent 10% of the total reaction volume (e.g. 5 µl in a 50 µl reaction).

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