



PRODUCT	SIZE	SKU
VitaScript™ FirstStrand cDNA Synthesis Kit	50 rxn / 20 µl	PCCSKU1301

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 primer and oligo-dTs in optimized buffer environment
Nuclease-free water	1 ml	

GENERAL CONSIDERATIONS

- High purity template RNA is essential for reliable efficient cDNA synthesis. A A₂₆₀ / A₂₈₀ 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.
- For the longest contiguous cDNA transcripts, anchored oligo-d(T)₁₈ primer mix are included in the VS reaction buffer. It ensures that the primers anneal to the start of the poly(A) tail and cover the entire gene.
- 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 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.

- 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 ₂ O	to 20 µl
total volume	20 µl

- As a negative control, replace VitaScript™ Enzyme Mix with 1 µl nuclease-free dH₂O.
- Incubate the 20 µl reaction for 1 hour at 42°C.
- Inactivate VitaScript™ Reverse Transcriptase for 10 minutes at 80°C. Dilute the reaction with nuclease-free dH₂O 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).