Xpert cDNA Synthesis Supermix (with gDNA eraser)

 # GK82.0100 (100 rxn)

(FOR RESEARCH ONLY)

Product Description

Presence of trace amounts of contaminating genomic DNA (gDNA) in RNA preparations may lead to significant problems, such as false-positive signals and misjudgment of gene expression levels, hence the effective removal of gDNA prior to cDNA synthesis is essential to ensure reliable results.

The Xpert cDNA Supermix (with gDNA eraser) combines an effective genomic DNA removal reaction mixture with a convenient Reverse Transcriptase mastermix (Xpert RTase Mix). Using gDNA eraser, any residual DNA (ssDNA, dsDNA and plasmid DNA) is eliminated efficiently from the RNA sample in just a few minutes. The reaction is stopped by adding a stop solution, eliminating the need of a heating or organic extraction step that could be prejudicial to the RNA integrity. The gDNA-free RNA solution can then be reverse-transcribed into cDNA directly, using the Xpert RTase Mix.

Xpert RTase Mix is an optimized mastermix containing a balanced concentration of oligo(dT) and random hexamer primers, dNTPs, RNase inhibitor, and Xpert Reverse Transcriptase (RNase H-). In this way, the need for multiple component additions is eliminated and thus the chance of handling errors is reduced, rendering excellent reproducibility and absolute convenience.

In summary, this system provides an efficient and fast method for the synthesis of high-quality, full-length (up to 8-10kb) first strand cDNA with excellent yields from purified poly(A)+ mRNA or total RNA templates.

Please note that, since the mastermix already contains primers, this product cannot be used with gene-specific primers. First strand cDNA can be directly used as template in PCR.

Kit Contents

Component	Volume (100 rxns)
Xpert RTase 5X Mix	400 µl
Xpert 4X Reaction Mix (gDNA eraser)	200 µl
Xpert 5X Reaction Stopper	200 µl
RNase-free water	1 ml

Quality Control

Functionally tested by cDNA synthesis and PCR amplification in comparison with previous batches. Absence of endonuclease-, exonuclease-, and nicking activity is verified on a lot-to-lot basis.

Storage

Store all components at -20ºC for up to 1 year.

Protocol

1. Mix the following components in a RNase-free microtube:

Component	Volume
template RNA	1ng - 2 µg total RNA or 1pg – 2ng poly(A)+ RNA
Xpert 4X Reaction Mix	2 µl
RNase free water	up to 8 µl

2. Using a thermocycler or thermoblock, incubate for 2 minutes at 42ºC
3. Add the following components to the mixture:

Component	Volume
Xpert 5X Reaction Stopper	2 µl

4. Mix and then add the following components to the mixture:

Component	Volume
Xpert RTase 5X Mix	4 µl
RNase free water	6 µl

5. Incubate at 25°C for 10 minutes.
6. Using a thermocycler or thermoblock , heat the microtube for
   a. 15 minutes at 42ºC (for usage in qPCR) or
   b. 50 minutes at 42ºC (for usage in PCR)

7. Inactivate Enzyme by heating for 5 min at 85ºC, then chill on ice.
8. Either use cDNA immediately as template in qPCR/PCR or store at -20ºC.

Notes

1. There is no need to purify poly(A)+ RNA from total RNA when using oligo$(dT)_{20}$ primers, however, doing so, may improve yield and overall purity of the final product.
2. One can prepare a no RT control by taking a small aliquot of the Xpert RTase 5X Mix and inactivate Xpert RTase by incubating at 85ºC for 5-10 minutes, prior to adding in step 4.