Xpert cDNA Synthesis Supermix

#GK86.0100 (100 rxns)
(FOR RESEARCH ONLY)

Product: 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.

Xpert cDNA Synthesis Supermix is an optimized mastermix containing a balanced concentration of oligo(dT) and random hexamer primers, dNTPs, 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. The Xpert cDNA Synthesis Supermix already includes gDNA eraser, for efficient elimination of any residual DNA (ssDNA, dsDNA and plasmid DNA) from the RNA sample that otherwise may lead to misjudgment of gene expression levels. Moreover, the Supermix also already includes GRiSP´s RNase inhibitor, which has an improved resistance to oxidation in comparison with human RNase inhibitors and is stable under even very low concentrations of DTT, making it an excellent choice for RNA protection.

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 and other downstream applications or stored for long-term at -20ºC.

Applications: cDNA synthesis

Contents: The Xpert cDNA Synthesis Supermix is sufficient for 100 reactions. The trial sample for 10 reactions.

Component	GK86.0100
Xpert cDNA Synthesis Supermix (5X)	400 µl
RNase-free water	1 ml

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

Storage: Store all components at -20ºC

Prior to use:

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. For more efficient gDNA removal from heavily contaminated samples, increase incubation time at 37°C to 30 minutes.

Usage:

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 cDNA Synthesis Supermix (5X)	4 µl
RNase-free water	up to 20 µl

2. Using a thermocycler or thermoblock, incubate for 15 minutes at 37°C, followed by 10 minutes at 60°C

3. (Optional) Stop the reaction by heating for 3 minutes at 95°C, followed by chilling on ice.

4. Either use cDNA immediately as template in qPCR/PCR or store at -20°C.