Xpert cDNA Synthesis Kit

 # GK80.0100 (100 rxn)

(FOR RESEARCH ONLY)

Product Description

The Xpert cDNA Synthesis Kit provides an efficient and fast method for the synthesis of high quality first strand cDNA from purified poly(A)+ mRNA or total RNA templates. The Xpert cDNA Synthesis core kit includes Xpert Reverse Transcriptase (RNase H-), a RNA-dependent DNA polymerase suitable for cDNA synthesis from long RNA templates. Xpert RTase has been optimized to perform under high temperatures (50°C-55°C), which facilitates the removal of secondary mRNA structures associated with high GC content. Together with the lack of RNase H activity, which ensures minimization of template degradation during long incubation times, this enables the preparation of long full-length cDNAs (up to 12kb).

Besides Xpert RTase, the Xpert cDNA Synthesis Kit also contains an RNase inhibitor, which has already been included, for protection of template degradation, reaction buffer, and dNTPs. GRiSP´s RNase inhibitor is 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. Furthermore, for convenience, both random primers (N6) and oligo(dT) primers are provided. The kit can also be used with gene-specific primers (GSP). First strand cDNA can be directly used as template in PCR.

Kit Contents

Component	Volume (100 rxns)
Xpert Reverse Transcriptase (200U/µl) (containing RNase Inhibitor (40U/µl)	100 µl
Random Hexamer primer (10 µM)	100 µl
Oligo(dT)$_{20}$ primer (10 µM)	100 µl
5x Reaction Buffer	400 µl
dNTP mix (10mM each)	100 µl
RNase-free water	2 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

Protocol

1. Thaw individual reagents thoroughly and mix briefly before use.
2. Mix the following components in a RNase-free microtube kept on ice:

Component	Volume
5x Reaction Buffer	4 µl
dNTP mix (10 mM each)	1 µl
primer (10 µM)	1 µl hexaprimer (N6) and/or 1 µl oligo(dT)$_{20}$
template RNA	1ng - 2 µg total RNA or 1pg – 2ng poly(A)+ RNA
Xpert RTase (200U/µl) (with RNase Inhibitor (40U/µl)	1 µl
RNase free water	up to 20 µl

3. Gently mix the reaction tube and then centrifuge briefly.

4. If using random primers, incubate at 25°C for 10min. (If using Oligo(dT) or GSP, skip this step).
5. Using a thermocycler or thermoblock, heat the microtube for
    a. 15 min at 50ºC-55ºC (for usage in qPCR) or
    b. 50 min at 50ºC-55ºC (for usage in PCR, for longer transcripts >9kb)

6. Inactivate RTase by heating for 5 min at 85ºC and chill on ice for 2 minutes.
7. Either use cDNA immediately as template in qPCR/PCR or store at -20ºC.

Notes

1. The RNA sample should be completely free of contaminating genomic DNA.
2. 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.
3. Whereas oligo(dT)$_{20}$ priming does not require any optimization, the ratio of random hexaprimer to RNA template influences strongly the average length of the synthesized cDNA: increasing the ratio will result in higher yield of shorter cDNA (<500bp), whereas decreasing the ratio will produce longer products. One can alter the ratio by varying the amount of template RNA.
4. One can prepare a no RT control by taking a small aliquot of the Xpert RTase and inactivate the enzyme by incubation at 85ºC for 5-10 minutes, prior to adding in step 2.

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