Xpert cDNA Synthesis Mastermix

 # GK81.0100 (100 rxn)

(FOR RESEARCH ONLY)

Product Description

Xpert Reverse Transcriptase (RNase H-) is a RNA-dependent DNA polymerase suitable for cDNA synthesis from long RNA templates. Xpert RTase has been optimized to perform under high temperatures (45°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, Xpert RTase enables the preparation of long full-length cDNAs (up to 15kb).

The Xpert cDNA Mastermix 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 Mastermix (2X) is an optimized reaction mix containing a balanced concentration of oligo(dT) and random hexamer primers, dNTPs, and RNase inhibitor. Note that, since the mastermix already contains primers, it 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 Reverse Transcriptase (200U/µl)	100 µl
Mastermix (2X)	1,2 ml
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 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
Mastermix (2X)	10 µl
RNase free water	up to 19 µl

2. Using a thermocycler or thermoblock, heat for 5 minutes at 65ºC (in order to remove possible secondary RNA structures), then place on ice for 2 minutes.
3. Add the following components to the mixture:

Component	Volume
Xpert RTase (200U/µl)	1,0 µl

4. Mix thoroughly and then centrifuge briefly
5. Incubate at 25°C for 10min
6. Using a thermocycler or thermoblock, heat the microtube for
    a. 15 min at 50ºC (for usage in qPCR) or
    b. 50 min at 50ºC (for usage in PCR)

7. Inactivate Enzyme by heating for 5 min at 85ºC
8. 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. One can prepare a no RT control by taking a small aliquot of the Xpert RTase and inactivate the enzyme by incubating at 85ºC for 5-10 minutes, prior to adding in step 3.

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