

Xpert cDNA Synthesis Kit

#GK80.0100 (100 rxns) | GK80 (trial size)
(FOR RESEARCH ONLY)



Product: 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 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.

Applications: cDNA synthesis

Contents: The Xpert cDNA Synthesis Kit contains sufficient reagents for 100 reactions. The trial sample is (GK80s) is sufficient for 10 reactions.

Component	GK80.0100
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

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 -20°C

Prior to use:

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 85°C for 5-10 minutes, prior to adding in step 2.

Usage:

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 50°C-55°C (for usage in qPCR) or
 - b. 50 min at 50°C-55°C (for usage in PCR, for longer transcripts >9kb)
6. Inactivate RTase by heating for 5 min at 85°C and chill on ice for 2 minutes.
7. Either use cDNA immediately as template in qPCR/PCR or store at -20°C.