

ab286905 – cDNA kit

For robust cDNA synthesis from any RNA template, DNA primer extension, dsDNA sequencing, producing RT PCR templates, generating hybridization probes and 3'-end duplex DNA labelling.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab286905

Storage and Stability

Store all components at -20°C in a non-frost-free freezer. Avoid repeated freeze-thaw cycles to retain maximum performance.

Materials Supplied

Item	Quantity 100 test kit	Storage Condition
Novo RTase	100 µl	-20°C
Oligo(dT) (10 µM)	100 µl	-20°C
Random Primers (10 µM)	100 µl	-20°C
dNTPs (10 mM)	100 µl	-20°C
5X RT Buffer	400 µl	-20°C
Nuclease-free H ₂ O	2 x 1 mL	-20°C

- *RNaseOFF Ribonuclease Inhibitor is already provided in the formulation of Novo RTase (200 U/µl) for 100 reactions kit

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- PCR Tubes
- Pipettes
- Water, Nuclease-free
- Primers (forward and reverse)
- Total RNA or poly(A) + mRNA

Assay Protocol

Δ Notes:

- RT-PCR should be assembled in a nuclease-free environment.
- RNA sample preparation, reaction mixture assembly, PCR, and subsequent reaction analysis should be performed in separate areas.
- The use of "clean", automatic pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Thoroughly thaw and mix individual components before use and assemble reactions on ice.

Components	Volume
Total RNA or poly(A) + mRNA	Variable
Primers	1 µl
dNTP Mix	1 µl
Water, Nuclease-free	Up to 20 µl
5X RT Buffer	4 µl
Novo RTase (200 U/µl)	1 µl

Δ Note: RNaseOFF Ribonuclease Inhibitor is already provided in the formulation of Novo RTase (200 U/µl) for the 100 reactions kit quantity

2. Gently mix the reaction and briefly centrifuge.
3. Perform cDNA synthesis by incubating the tube for either 15 min at 50-55°C.
4. Optional: Stop the reaction by heating it at 85°C for 5 min. Chill on ice. The newly synthesized first strand cDNA is ready for immediate downstream applications, or for long-term storage at -20°C.

General Information

- Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
- For longer transcripts > 9 kb, yields can be increased by incubating at 50-55°C for 30-50 min.
- RNA samples must be free of genomic DNA contamination.
- The ratio of Random Primers to RNA is often critical in terms of the average length of cDNA synthesized. A higher ratio of Random Primers to RNA will result in a higher yield of shorter (~500 bp) cDNA, whereas a lower ratio will lead to longer cDNA products. Due to the lower annealing temperature of Random Primers, incubate at 25 °C for 10 min to allow for primer annealing prior to reverse transcription.
- To remove RNA complementary to the cDNA, add 1 µl (2 U) of E. coli RNase H and incubate at 37 °C for 20 min.

Technical Support

Copyright © 2024 Abcam. All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)