RT Ace First Strand cDNA Synthesis Kit

This product is intended for research use only. Do not use this product for diagnostic purposes.

Made in SAUDI ARABIA
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Principle and Intended use

This product is intended for first strand complementary DNA synthesis from RNA templates. It is suitable for qPCR-based studies such as gene expression, Sanger sequencing, etc. The product is specifically made for research purposes only, please do not use it in diagnostic applications.

Our kit works by synthesizing cDNA from an RNA template in an RNA-dependent DNA polymerization reaction, or reverse transcription. The RT Ace Reverse

Transcriptase, an M-MuLV mutant that is thermally stable up to 65°C, has the ability to synthesize full size cDNA up to 20kb.

In order to combat RNase contamination during cDNA synthesis, we supply our RT Ace enzyme premixed with murine RNase inhibitor in optimized concentrations.

Kit contents and storage conditions

This kit contains 6 tubes as indicated in the table below. Store at -20°C immediately upon receipt.

Instructions for the RNA template

1. When working with RNA, ensure to use RNase-free equipment, plastics, and solutions in the entirety of the workflow.

2. The RNA amount to be reverse-transcribed could range from 5pg-5µg.

3. For gene expression studies, it is advisable to reverse transcribe an equal amount of RNA for each sample to minimize possible variations due to the different concentrations of the templates. This can be achieved by unifying the RNA concentration for all samples prior to cDNA synthesis.

4. It is advisable to use a high-quality RNA template (RIN≥7). To achieve this, make sure to use RNA that is freshly extracted, or that was stored at -80°C for less than a year.

   a. For RNA of lower quality (RIN<7) due to long storage or that was extracted from FFPE tissue, designing primers whose PCR product is relatively small (~60-80bp) may yield more consistent results.

5. Make sure your RNA is of high purity as determined by spectrophotometry, i.e., 260/280 ratio of ~2.0, and 260/230 ratio of ~2.0–2.2.

Protocol

1. **Thaw all kit components and RNA samples completely at room temperature then put them on ice. Keep working on ice whenever applicable.**

2. **Prepare a reaction mix sufficient for the total number of samples plus 10% to account for pipetting errors; this is achieved by mixing the following components for a final volume of 20µL (after adding the RNA template in step4):**

Component	Volume per reaction	***Example:*** ***Volume per 10+1 reactions***
RT Ace Reverse Transcriptase	1 µL	*11 µL*
RT Ace Buffer (5X)	4 µL	*44 µL*
dNTP Mix (20X)	1 µL	*11 µL*
Random hexamers (20X)* OR Gene-specific primers (2µM)	1 µL random hexamers OR 1 µL of specific primers	*11 µL random hexamers* *OR* *5.5 µL of specific primers*
RNase-free water	3 µL	*33 µL*

*** The poly(dT)$_{20}$VN and random hexamers could be used together by adding 0.5µL each**

3. **Load 10µL of the reaction mix into the allocated PCR plate wells or PCR tubes.**

5. **Heat the RNA samples to 70°C for 2 minutes to reduce RNA folding then place immediately on ice. Load 10µL of RNA into each allocated well/PCR tube.**

Note: if lower volumes of RNA are to be added, fill the reaction up to 20µLwith RNase-free water

5 **Program the thermal cycler as follows. After the reaction is terminated, use the resulting cDNA samples immediately or store them at -20°C.**

Step	Temperature	Time	Cycles
Only when random hexamers are used	25°C	5 min	Hold
Reverse transcription	50°C*	30 min**	Hold
Enzyme destruction	85°C	5 min	Hold

*** Could be increased up to 60°C for GC-rich templates**
*** Could be increased up to 60 min for low input RNA**

E Support

Support

For questions, suggestions, or technical support, feel free to contact us by email on: support@havensci.com

F Quality Control

Quality Control

Each lot is tested against predetermined parameters to ensure consistent performance between all lots.

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RT Ace First Strand cDNA Synthesis Kit
Product Manual (PCR3005 – PCR3105)
Version 4.0
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