ab185916 – Hi-Fi cDNA Synthesis Kit

Instructions for Use

For cDNA synthesis from various RNA samples

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Effective and efficient preparation of cDNA from RNA can help ensure reliable data in various downstream applications including real-time qPCR and RNA/cDNA-sequencing. Hi-Fi cDNA Synthesis Kit (ab185916) has been optimized and validated for cDNA synthesis from various RNA samples, particularly from bisulfite-converted RNA and enriched RNA fragments. The kit contains all necessary components including a highly sensitive and thermostable recombinant reverse transcriptase which provides enhanced cDNA synthesis efficiency, high fidelity, and a wide range of activity for varying amounts of RNA templates (0.1 ng to 2 µg). Due to its high thermal stability, low RNase activity and resistance to inhibition by rRNA and tRNA, the recombinant RT included in the kit can be used for synthesis of long cDNA products at temperatures from 42°C-60°C using total RNA, and GC-rich templates such as bisulfite converted RNA. A recombinant RNase inhibitor is also included in the kit to prevent the degradation of target RNA due to ribonuclease contamination.

Abcam's Hi-Fi cDNA Synthesis Kit (ab185916) is optimized to synthesize a DNA copy specifically using bisulfite-converted RNA and enriched RNA fragments. The kit is also suitable for cDNA synthesis using total RNA. The synthesized DNA can then be used for a variety of downstream applications including PCR and cDNA library construction. The RT enzyme included in the kit has low intrinsic RNase activity, which delivers the highest reverse transcription accuracy currently available and promotes full-length cDNA synthesis.

2. ASSAY SUMMARY

Start with RNA isolated from various tissue or cell samples

Incubate with Random Primer and dNTP Mix

Heat then place on ice

Incubate with RT Reaction buffer, DTT, RNAase Inhibitor and RT Enzyme Mix

Heat then store at -20°C

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

5. MATERIALS SUPPLIED

Item	20 Reactions	Storage Condition (Before Preparation)
5x RT Reaction Buffer	100 µL	–20°C
10 mM dNTP Mix	25 µL	–20°C
DTT (100mM)	50 µL	–20°C
RNAase Inhibitor	25 µL	–20°C
Random Primer	25 µL	–20°C
RT Enzyme Mix	25 µL	–20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Adjustable pipette or multiple-channel pipette
- Multiple-channel pipette reservoirs
- Aerosol resistant pipette tips
- Thermocycler without heated lid
- 1.5 ml microcentrifuge tubes
- 0.2 mL PCR tubes
- RNA sample

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Complete removal of all solutions and buffers during wash steps.
- **This kit is sold based on number of reactions. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

All reagents supplied ready to use.

10. SAMPLE PREPARATION

Input Amount: RNA amount can range from 0.1 ng to 2 µg per reaction. An optimal amount is 200–500 ng per reaction. Starting RNA may be in water or in a buffer such as TE. RNA should be high quality and relatively free of DNA. DNAse I can be used to remove DNA and RNA should be eluted in RNase-free water.

RNA Storage: RNA should be stored at -20°C or -80°C until use.

11. ASSAY PROCEDURE

cDNA Synthesis

11.1 Add the following in a 0.2 mL PCR tube on ice:

Component	Amount (µL)
RNA (200-500 ng)	10
Random Primer	1
10 mM dNTP Mix	1

11.2 Heat in a thermocycler (no heated lid) at 65° for 3 minutes. Place on ice immediately for at least 1 minute.

11.3 Add the following to the tube on ice final volume 20 µL;

Component	Amount (µL)
5X RT Reaction Buffer	4
DTT (100mM)	2
RNase Inhibitor	1
RT Enzyme Mix	1

11.4 Vortex the sample briefly to mix and collect by centrifugation. Incubate as follows: 42°C for 45 minutes followed by 80°C for 5 minutes (no heated lid).

11.5 Store the cDNA synthesis reaction at -20°C, or proceed directly to the next application such as qPCR (section 12 Working with qPCR) or RNA/cDNA-sequencing.

12. ANALYSIS

qPCR Method

When working with qPCR, we recommend using a hot start polymerase system and has been optimized to decrease the overall qPCR amplification time. The master mix is provided at 2X concentration for easier preparation of PCR reactions requiring only the addition of primers and templates. With this kit, the qPCR can be finished in as short as 70 minutes.

Prepare the PCR Reactions

Component	Volume (µL)	Final concentration
Master Mix (2X)	10	1X
Forward Primer	1	0.4-0.5 µM
Reverse Primer	1	0.4-0.5 µM
DNA Template	1-2	50 pg – 0.1 µg
DNA/RNA free H2O	6-7	
Total volume	20	

For the negative control, use RNAase free water instead of cDNA template.

Program the PCR Reactions:

Cycle step	Temperature	Time	Cycle
Activation	95 °C	7 minutes	1X
Cycling	95 °C	10 seconds	40-45
	55 °C	10 seconds	
	72 °C	8 seconds	
Final extension	72 °C	1 minute	1

13. TROUBLESHOOTING

Problem	Cause	Solution
Little or no cDNA synthesis	Poor RNA quality (RNA is severely degraded)	Check if the sample RNA 260/280 ratio is between 1.9-2.0. Analyze RNA on a denaturing gel to verify RNA integrity
	RT inhibitor is contained in RNA	The common RNA inhibitors such as SDS, EDTA and formamide can be removed by re-precipitation and clean-up of RNA with ethanol
	Temperature is incorrect	Check if the temperature is appropriate for cDNA synthesis
	Insufficient starting RNA amount	Increase the amount of starting RNA, especially for amplifying low-copy genes from total RNA
	Kit is not stored or handled properly	Store all components of the kit at -20°C
Poor specificity in qPCR	Non-specific primers	PCR primers were not appropriate or were incorrectly designed. Ensure the primers are specific for the target genes
	Genome DNA contamination	Treat RNA with DNase I and re-purify

14. NOTES

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