High Capacity cDNA Reverse Transcription Kit

USER GUIDE

For 200 and 1000 reaction kits

Catalog Numbers 4368813, 4368814, 4374966, 4374967

Publication Number MAN0017977

Revision A.0

Manufacturer: Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, THERMO FISHER SCIENTIFIC INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Revision history: Revision history of Pub. no. MAN0017977

Revision	Date	Description
A.0	16 July 2018	Baseline for this revision history.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Trademarks: All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. TaqMan is a registered trademark of Roche Molecular Systems, Inc., used under permission and license.

Contents

Product information

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Product description

The Applied Biosystems™ High-Capacity cDNA Reverse Transcription Kit contains all the reagents needed for reverse transcription (RT) of up to 2 µg of total RNA to single-stranded cDNA in a 20 µL reaction. The cDNA prepared using the kit is suitable for quantitative PCR, archival storage, or conversion to cRNA.

The random primer method is used for initiating cDNA synthesis, and ensure that first strand synthesis occurs efficiently with all species of RNA molecules present, including mRNA and rRNA. The kit has been tested extensively against various RNA templates, including G/C-rich and A/U-rich RNA species. The effect of relative mRNA abundance was also examined to see if the reverse transcriptase reaction generates products in a manner directly dependent to the amount of input RNA template. In all cases, quantitative conversion of mRNA and 18S ribosomal RNA species was observed.

Available kits

Kit	Cat. No.
High-Capacity cDNA Reverse Transcription Kit, 200 reactions	4368814
High-Capacity cDNA Reverse Transcription Kit, 1000 reactions	4368813
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, 200 reactions	4374966
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, 1000 reactions	4374967

Contents and storage

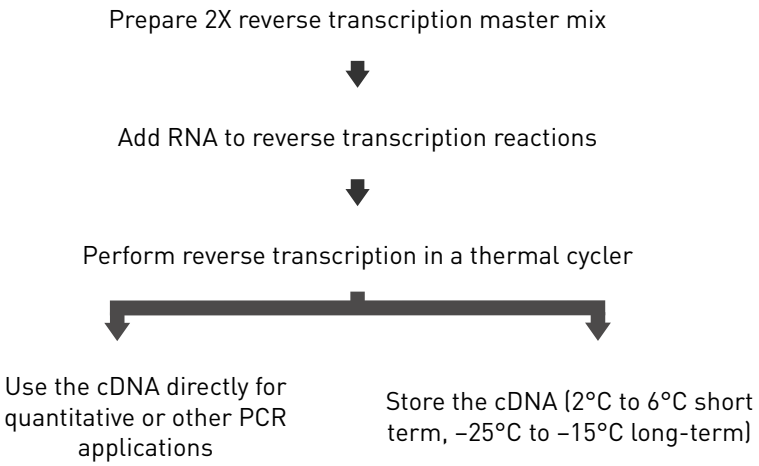
Contents	Cat. No.				Storage
	4368813	4374967	4368814	4374966	
10X RT Buffer	2 × 1.0 mL	2 × 1.0 mL	1 × 1.0 mL	1 × 1.0 mL	–25°C to –15°C
10X RT Random Primers	2 × 1.0 mL	2 × 1.0 mL	1 × 1.0 mL	1 × 1.0 mL	
25X dNTP Mix (100 mM)	1 × 1.0 mL	1 × 1.0 mL	1 × 0.2 mL	1 × 0.2 mL	
MultiScribe™ Reverse Transcriptas (50 U/µL)	1 × 1.0 mL	1 × 1.0 mL	2 × 0.1 mL	2 × 0.1 mL	
RNase Inhibitor	—	5 × 200 µL	—	1 × 200 µL	

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
Nuclease-free water	MLS
Pipette tips, aerosol-resistant	MLS
Pipettes, positive-displacement	MLS
Vortexer	MLS
Microcentrifuge	MLS
Disposable gloves	MLS
Centrifuge (>3,000 × *g* (rcf), temperature controlled)	MLS
Reagent Tubes with Caps, 10-mL	MLS

Workflow


Prepare 2X reverse transcription master mix
Add RNA to reverse transcription reactions
Perform reverse transcription in a thermal cycler
Use the cDNA directly for quantitative or other PCR applications
Store the cDNA (2°C to 6°C short term, –25°C to –15°C long-term)

Methods

Guidelines for template RNA

Observe the following guidelines to ensure optimal performance when using the High-Capacity cDNA Reverse Transcription Kit.

- Use up to 2 µg of total RNA per 20-µL reaction.
- Dissolve RNA in PCR-compatible buffer or water.
- Ensure RNA is free of inhibitors of reverse transcription and PCR.
- Ensure RNA is free of RNase activity. If you suspect that the RNA contains RNase activity, add RNase Inhibitor to the reverse transcription reaction at a final concentration of 1 U/µL.

Reverse transcription reaction guidelines

The kit contains reagents that, when combined, form a 2X reverse transcription (RT) master mix. An equal volume of RNA sample should be added. To avoid RNase contamination, RNase-free reagents and consumables must be used.

Prepare the 2X RT master mix (20-µL reaction)

1. Allow the kit components to thaw on ice.
2. Calculate the volume of components needed to prepare the required number of reactions.

   Note: Prepare the RT master mix on ice.

Component	Volume	
	with RNase Inhibitor	without RNase Inhibitor
10X RT Buffer	2.0 µL	2.0 µL
25X dNTP Mix (100 mM)	0.8 µL	0.8 µL
10X RT Random Primers	2.0 µL	2.0 µL
MultiScribe™ Reverse Transcriptase	1.0 µL	1.0 µL
RNase Inhibitor	1.0 µL	—
Nuclease-free H_2O	3.2 µL	4.2 µL
Total per reaction	10.0 µL	10.0 µL

   IMPORTANT! Include additional reactions in the calculations to provide excess volume for the loss that occurs during reagent transfers.

3. Place the 2X RT master mix on ice and mix gently.

Prepare the reverse transcription reactions

1. Pipette 10 µL of 2X RT master mix into each well of a 96-well reaction plate or individual tube.
2. Pipette 10 µL of RNA sample into each well, pipetting up and down two times to mix.
3. Seal the plates or tubes.
4. Briefly centrifuge the plate or tubes to spin down the contents and to eliminate any air bubbles.
5. Place the plate or tubes on ice until you are ready to load the thermal cycler.

Perform reverse transcription

1. Program the thermal cycler using the conditions below.

   IMPORTANT! These conditions are optimized for use with the High-Capacity cDNA Reverse Transcription Kit.

Settings	Step 1	Step 2	Step 3	Step 4
Temp.	25°C	37°C	85°C	4°C
Time	10 minutes	120 minutes	5 minutes	Hold

2. Set the reaction volume to 20 µL.
3. Load the reaction plates or tubes into the thermal cycler.
4. Start the thermal cycler run.

PCR good laboratory practices

Good laboratory practices for PCR and RT-PCR

When preparing samples for PCR or RT-PCR amplification:

- Wear clean gloves and a clean lab coat.
  - Do not wear the same gloves and lab coat that you have previously used when handling amplified products or preparing samples.
- Change gloves if you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation and reaction setup.
  - Amplification and analysis of products.
- Do not bring amplified products into the reaction setup area.
- Open and close all sample tubes carefully. Avoid splashing or spraying samples.
- Keep reactions and components capped as much as possible.
- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Clean lab benches and equipment periodically with 10% bleach solution or DNA decontamination solution.

B Results from reverse transcription

Quantitative PCR

To determine the yield of the cDNA from the reverse transcription of total RNA, use quantitative PCR to test various input amounts of RNA for the cDNA yield of different gene targets.

The following table lists some targets and available Applied Biosystems™ kits that can be used to evaluate the yield of cDNA conversion.

Gene target	Kit name	Cat. No.
18S	TaqMan® Ribosomal RNA Control Reagents	4308329
GAPDH	TaqMan® GAPDH Control Reagents (Human)	402869
GAPDH	TaqMan® Rodent GAPDH Control Reagents	4308313
ß-actin	TaqMan® ß-actin Detection Reagents	401846

Other TaqMan® Gene Expression Assays can be used to evaluate the yield of cDNA conversion. For a list of available assays, go to **thermofisher.com**.

Yields for different targets

For the example in this appendix, the input total RNA was obtained from human Raji cells, and the RNA was converted to cDNA using the High-Capacity cDNA Reverse Transcription Kit. Figure 1 shows an example of quantitative PCR results from the cDNA for 11 different gene targets, which vary in expression levels.

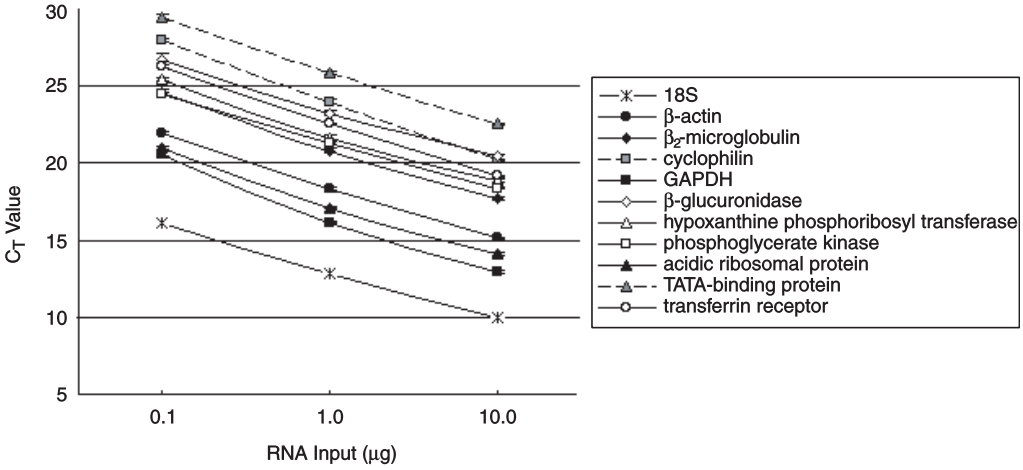


Figure 1 The expected ΔCT values of 3.3 for each tenfold increase in the RNA input quantity are obtained for 11 different RNA transcripts converted to cDNA from different input quantities of total RNA.

Note: The amplicon for β2-microglobulin in this study was specifically designed to be A/U rich. The threshold cycle (CT) values are plotted against RNA input amounts of 0.1, 1.0, and 10.0 µg in 100-µL reactions.

Yields for different targets

To achieve optimal conversion, allow reverse transcription to occur for 120 minutes at 37 °C. Figures 2, 3, and 4 show CT values plotted against reaction time in minutes for three different targets (18S, GAPDH, and β2-microglobulin) and three input amounts of RNA (0.1, 1.0, and 10.0 µg in 100-µL reactions).

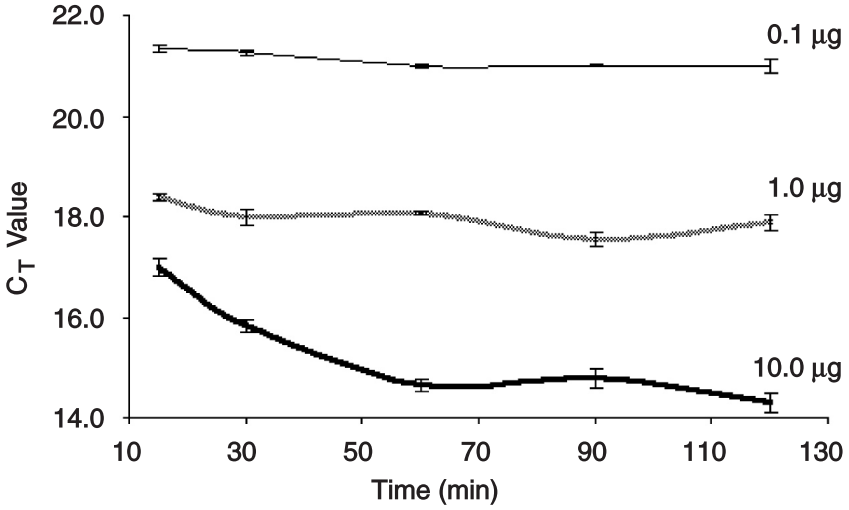


Figure 2 The rate of conversion of 18S RNA to cDNA reaches a maximum at 120 minutes with 10 µg of input RNA and at 30 minutes or less with 0.1 to 1.0 µg of input RNA (based on 100-µL reactions).

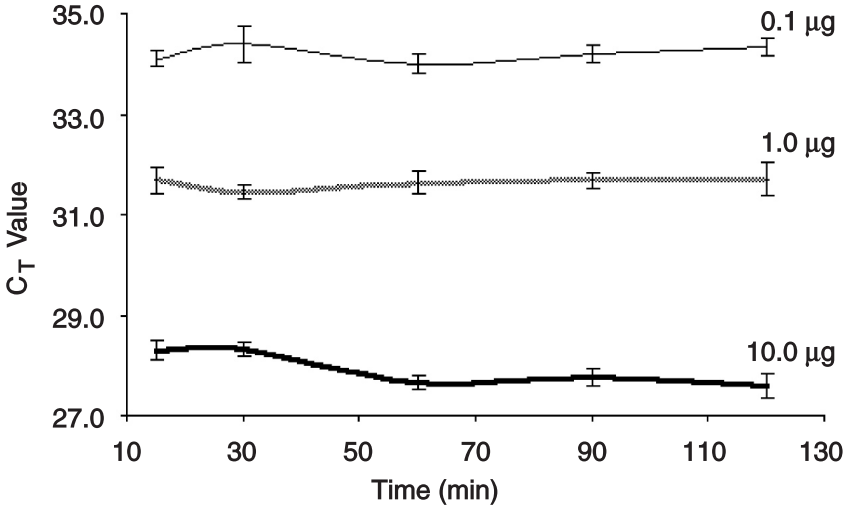


Figure 3 The rate of conversion of GAPDH RNA to cDNA reaches a maximum at 60 minutes or less at all RNA input levels (based on 100-µL reactions).

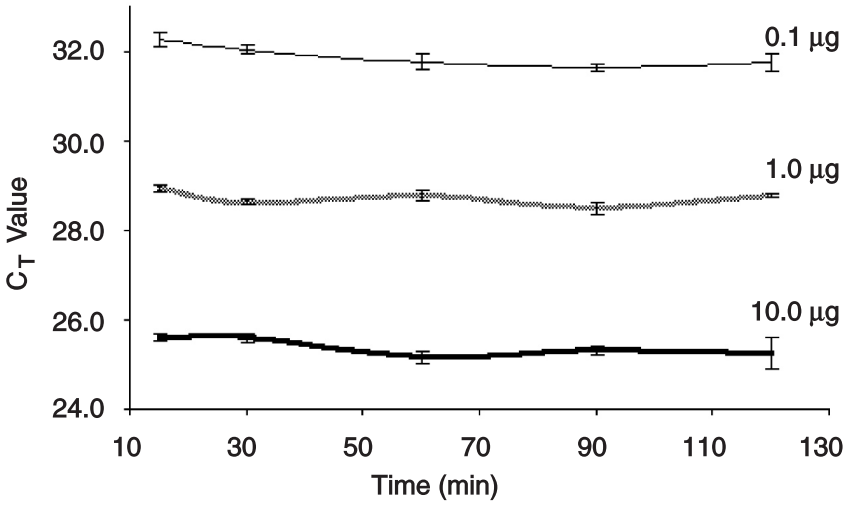


Figure 4 The rate of conversion of β2-microglobulin RNA to cDNA reaches a maximum at 60 minutes or less at all RNA input levels (based on 100-µL reactions).

C

Accessory products

Accessory products

Unless otherwise indicated, all materials are available through **thermofisher.com**. MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Item	Source
MicroAmp™ Fast Optical 96-Well Reaction Plate, 0.1 mL	4346907
MicroAmp™ Fast Optical 96-Well Reaction Plate with Barcode, 0.1 mL	4346906
MicroAmp™ Optical 96-Well Reaction Plate	N8010560
MicroAmp™ 8-Tube Strip, 0.2 mL	N8010580
MicroAmp™ Fast 8-Tube Strip, 0.1 mL	4358293
MicroAmp™ 8-Cap Strip, clear	N8010535
MicroAmp™ Clear Adhesive Film	4306311
MicroAmp™ Optical Adhesive Film	4360954
MicroAmp™ Optical 96-Well Reaction Plate with Barcode & Optical Caps	403012
MicroAmp™ Optical 8-Cap Strips	4323032
MicroAmp™ Cap Installing Tool	4330015
MicroAmp™ Adhesive Film Applicator	4333183
RNase Inhibitor [1]	N8080119

[1] Included with Cat. Nos. 4374966 and 4374967

D

Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  www.cdc.gov/biosafety/publications/bmbl5/BMBL.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.