High Capacity cDNA Reverse Transcription Kits

For 200 and 1000 Reactions

Protocol

Applied Biosystems

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Contents

Preface

This preface covers:

Safety

Safety Alert Words

Four safety alert words appear in Applied Biosystems user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT, CAUTION, WARNING, DANGER**—implies a particular level of observation or action, as defined below.

Definitions

IMPORTANT! – Indicates information that is necessary for proper instrument operation, accurate chemistry kit use, or safe use of a chemical.

CAUTION – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

WARNING – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

DANGER – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.

Chemical Hazard Warning

WARNING **CHEMICAL HAZARD.** Some of the chemicals used with Applied Biosystems instruments and protocols are potentially hazardous and can cause injury, illness, or death.

Chemical Safety Guidelines

To minimize the hazards of chemicals:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "About MSDSs" on page vi.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the MSDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the MSDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

About MSDSs

Chemical manufacturers supply current Material Safety Data Sheets (MSDSs) with shipments of hazardous chemicals to *new* customers. They also provide MSDSs with the first shipment of a hazardous chemical to a customer after an MSDS has been updated. MSDSs provide the safety information you need to store, handle, transport, and dispose of the chemicals safely.

Each time you receive a new MSDS packaged with a hazardous chemical, be sure to replace the appropriate MSDS in your files.

Obtaining MSDSs

The MSDS for any chemical supplied by Applied Biosystems is available to you free 24 hours a day. To obtain MSDSs:

1. Go to **https://docs.appliedbiosystems.com/msdssearch.html**
2. In the Search field of the MSDS Search page:
   a. Type in the chemical name, part number, or other information that you expect to appear in the MSDS of interest.
   b. Select the language of your choice.
   c. Click **Search**.

3. To view, download, or print the document of interest:
    a. Right-click the document title.
    b. Select:
        - **Open** – To view the document
        - **Save Target As** – To download a PDF version of the document to a destination that you choose
        - **Print Target** – To print the document
4. To have a copy of an MSDS sent by fax or e-mail, in the Search Results page:
    a. Select **Fax** or **Email** below the document title.
    b. Click **RETRIEVE DOCUMENTS** at the end of the document list.
    c. Enter the required information.
    d. Click **View/Deliver Selected Documents Now**.

Note: For the MSDSs of chemicals not distributed by Applied Biosystems, contact the chemical manufacturer.

Chemical Waste Hazards

HAZARDOUS WASTE. Refer to Material Safety Data Sheets and local regulations for handling and disposal.

CHEMICAL WASTE HAZARD. Wastes produced by Applied Biosystems instruments are potentially hazardous and can cause injury, illness, or death.

CHEMICAL STORAGE HAZARD. Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

Chemical Waste Safety Guidelines

To minimize the hazards of chemical waste:

- Read and understand the Material Safety Data Sheets (MSDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide 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.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the MSDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).For additional safety guidelines, consult the MSDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste Disposal

If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument 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

BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; **http://bmbl.od.nih.gov**)
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; **http://www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**).
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

Additional information about biohazard guidelines is available at:

http://www.cdc.gov

How to Obtain Support

For the latest services and support information for all locations, go to **http://www.appliedbiosystems.com**, then click the link for **Support**.

At the Support page, you can:

- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Order Applied Biosystems user documents, MSDSs, certificates of analysis, and other related documents
- Download PDF documents
- Obtain information about customer training
- Download software updates and patches

In addition, the Support page provides access to worldwide telephone and fax numbers to contact Applied Biosystems Technical Support and Sales facilities.

High Capacity cDNA Reverse Transcription Kits Protocol

Overview

Purpose of the High Capacity cDNA Reverse Transcription Kits

The High Capacity cDNA Reverse Transcription Kits for 200 and 1000 reactions contain all the reagents needed for reverse transcription (RT) of total RNA to single-stranded cDNA using a reaction size of 20 µL.

Kit Features

Use the kit for:

- Quantitatively converting up to 2 µg (for a 20-µL reaction) of total RNA to cDNA
- Generating single-stranded cDNA suitable for quantitative PCR applications
- Generating single-stranded cDNA suitable for short- or long-term storage

Output Applications

The cDNA reactions prepared using the High Capacity cDNA Reverse Transcription Kits can be used in a variety of applications, including:

- Quantitative PCR
- Archival storage
- Conversion to cRNA

About This Protocol

This protocol describes:

- Procedures for using the kits
- Recommendations for using the cDNA created using the kits
- Examples of cDNA conversion performance obtained using the kits

Good Laboratory Practices

PCR Good Laboratory Practices

PCR assays require special laboratory practices to avoid false positive amplifications (Kwok and Higuchi, 1989). The high throughput and repetition of these assays can lead to amplification of a single DNA molecule (Saiki *et al.,* 1985; Mullis and Faloona, 1987).

- Wear a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation) and clean gloves when preparing samples for PCR amplification.
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas, dedicated equipment, and supplies for:
  - Sample preparation and PCR setup
  - PCR amplification and post-PCR analysis
- Never bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes and reaction plates carefully. Do not splash or spray PCR samples.
- Keep reactions and components sealed as much as possible.
- Use positive-displacement pipettes or aerosol-resistant pipette tips.
- Clean lab benches and equipment periodically with freshly diluted 10% chlorine bleach solution.

Bibliography

Kwok, S. and Higuchi, R. 1989. Avoiding false positives with PCR. *Nature* 339:237-238.

Mullis, K.B. and Faloona, F.A. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. *Methods Enzymol*. 155:335-350.

Saiki, R.K., Scharf, S., Faloona,F., *et al.,* 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 230:1350-1354.

Materials and Equipment

Kit Types

Table 1 lists the four available kit sizes.

Table 1 Available kits

Kit Name	Applied Biosystems Part Number
High Capacity cDNA Reverse Transcription Kit, 1000 reactions	4368813
High Capacity cDNA Reverse Transcription Kit, 200 reactions	4368814
High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, 1000 reactions	4374967
High Capacity cDNA Reverse Transcription Kit with RNase Inhibitor, 200 reactions	4374966

Kit Components

Table 2 lists all the kit components.

Table 2 Kit components

Component	Quantity in 1000 reaction kit	Quantity in 200 reaction kit
10× RT Buffer, 1.0 mL	2 tubes	1 tube
10× RT Random Primers, 1.0 mL	2 tubes	1 tube
25× dNTP Mix (100 mM)	1 tube, 1.0 mL	1 tube, 0.2 mL
MultiScribe™ Reverse Transcriptase, 50 U/µL	1 tube, 1.0 mL	2 tubes, 0.1 mL
RNase Inhibitor‡, 100 µL	10 tubes	2 tubes

‡ Only in orders that include RNase inhibitor (PN 4374966 or 4374967).

Kit Storage

Store all kit components at –15 to –25 °C.

Equipment Required but Not Supplied

Table 3 lists the equipment required to use the High Capacity cDNA Reverse Transcription Kits.

Table 3 Required equipment

Equipment	Source
Thermal cycler (one of the following): • Applied Biosystems 9800 Fast Thermal Cycler with 96-Well Aluminum Sample Block Module • GeneAmp® PCR System 9700 Thermal Cycler • GeneAmp® PCR System 9600 Thermal Cycler	Applied Biosystems (PN 4352604) See your Applied Biosystems sales representative
Centrifuge with 96-well adapter	Major laboratory supplier (MLS)
Microcentrifuge	MLS
Vortexer	MLS

Materials Required but Not Supplied

Table 4 lists the non-kit materials required to use the High Capacity cDNA Reverse Transcription Kits.

Table 4 Required materials

Materials/Consumables	Source
MicroAmp™ Fast 96-Well Reaction Plate	Applied Biosystems (PN 4346907)
MicroAmp™ Fast 96-Well Reaction Plate with Bar Code	Applied Biosystems (PN 4346906)
MicroAmp™ Optical 96-Well Reaction Plate	Applied Biosystems (PN N8010560)
MicroAmp™ 8-tube strip	Applied Biosystems (PN N8010580)
MicroAmp™ Fast 8-tube strip	Applied Biosystems (PN 4358203)
MicroAmp™ 8-cap strip	Applied Biosystems (PN N8010535)

Table 4 Required materials *(continued)*

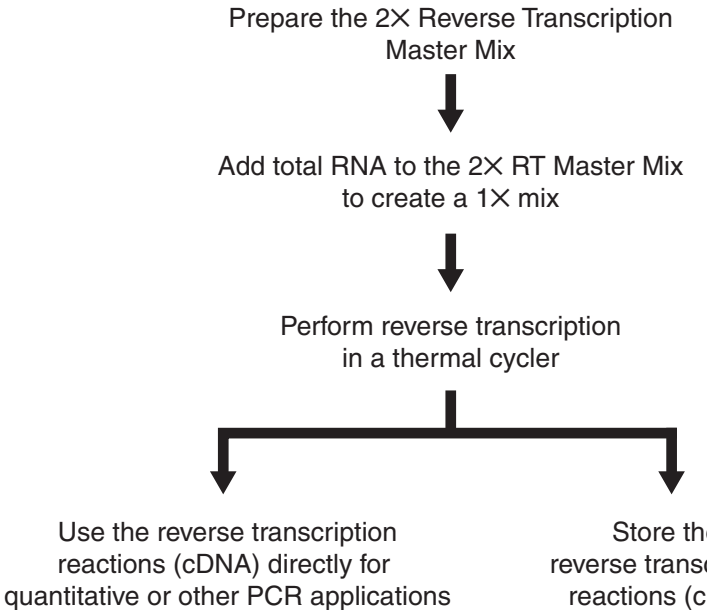
Materials/Consumables	Source
Clear adhesive film	Applied Biosystems (PN 4306311)
Optical adhesive film	Applied Biosystems (PN 4360954)
MicroAmp™ Optical 96-Well Reaction Plates and Optical Caps	Applied Biosystems (PN 403012)
MicroAmp™ Optical Caps, 8 Caps/Strip	Applied Biosystems (PN 4323032)
Reagent Tubes with Caps, 10-mL	Applied Biosystems (PN 4305932)
RNase Inhibitor‡ (for kits PN 4368813 and 4368814)	Applied Biosystems (PN N8080119)
Nuclease-free H_2O	MLS
Pipette tips, aerosol-resistant	MLS
Pipettors, positive-displacement	MLS
Disposable gloves	MLS
Cap Installing Tool	Applied Biosystems (PN 4330015)
Adhesive Seal Applicator	Applied Biosystems (PN 4333183)

‡ Included with kits PN 4374966 and 4374967.

Using the High Capacity cDNA Reverse Transcription Kits

Overview

To synthesize single-stranded cDNA from total RNA using the High Capacity cDNA Reverse Transcription Kits:



RNA Template Guidelines

For optimal performance of the High Capacity cDNA Reverse Transcription Kits, Applied Biosystems recommends using RNA that is:

- Free of inhibitors of reverse transcription and PCR
- Dissolved in PCR-compatible buffer or water
- Free of RNase activity

Note: If you suspect that the RNA contains RNase activity, add RNase Inhibitor to the reverse transcription reaction at a final concentration of 1.0 U/µL.

Input Amount of Total RNA

Use up to 2 µg of total RNA per 20-µL reaction.

Preparing the 2× Reverse Transcription Master Mix

Prepare the 2× RT master mix using the kit components before preparing the reaction plate.

To prepare the 2× RT master mix (per 20-µL reaction):

1. Allow the kit components to thaw on ice.

2. Referring to the table below, calculate the volume of components needed to prepare the required number of reactions.

   Note: Prepare the RT master mix on ice.

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

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

   WARNING **CHEMICAL HAZARD. 10× Reverse Transcription Buffer** may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate eyewear, clothing, and gloves.

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

Preparing the cDNA Reverse Transcription Reactions

To prepare the cDNA RT reactions:

1.	Pipette 10 µL of 2× 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.

Performing Reverse Transcription

To perform reverse transcription:

1. Program the thermal cycler conditions using one of the thermal cyclers listed in Table 3 on page 4.

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

	Step 1	Step 2	Step 3	Step 4
Temperature (°C)	25	37	85	4
Time	10 min	120 min	5 min	∞

2. Set the reaction volume to **20 µL**.
3. Load the reactions into the thermal cycler.
4. Start the reverse transcription run.

Storing cDNA Reverse Transcription Reactions

You can store cDNA RT plates or tubes prepared using the High Capacity cDNA Reverse Transcription Kits for short-term or long-term storage.

Storage Duration	Storage Temperature (°C)
Short-term (up to 24 hours before use)‡	2 to 6
Long-term	−15 to −25

‡ For prolonged storage at 2 to 6 °C, add EDTA to a final concentration of 1 mM to chelate cations and to prevent nucleic acid degradation.

IMPORTANT! If required, briefly centrifuge the archive plates or tubes before storing to spin down the contents and to eliminate any air bubbles.

Appendix A: Examples of cDNA Yields 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 table below lists some targets and Applied Biosystems kits that you can use to evaluate the yield of cDNA conversion.

Gene Target	Kit	Applied Biosystems Part Number
18S	TaqMan® Ribosomal RNA Control Reagents	4308329
GAPDH	TaqMan® GAPDH Control Reagents [Human]	402869
GAPDH	TaqMan® Rodent GAPDH Control Reagents	4308313
β-actin	TaqMan® β-actin Detection Reagents	401846

You can use other TaqMan® Gene Expression Assays to evaluate the yield of cDNA conversion. For a list of available assays, visit **www.appliedbiosystems.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 Kits.

Figure 1 shows an example of quantitative PCR results from the cDNA for 11 different gene targets, which vary in expression levels.

Note: The amplicon for β_2-microglobulin in this study was specifically designed to be A/U rich.

The threshold cycle (C_T) values are plotted against RNA input amounts of 0.1, 1.0, and 10.0 µg in 100-µL reactions.

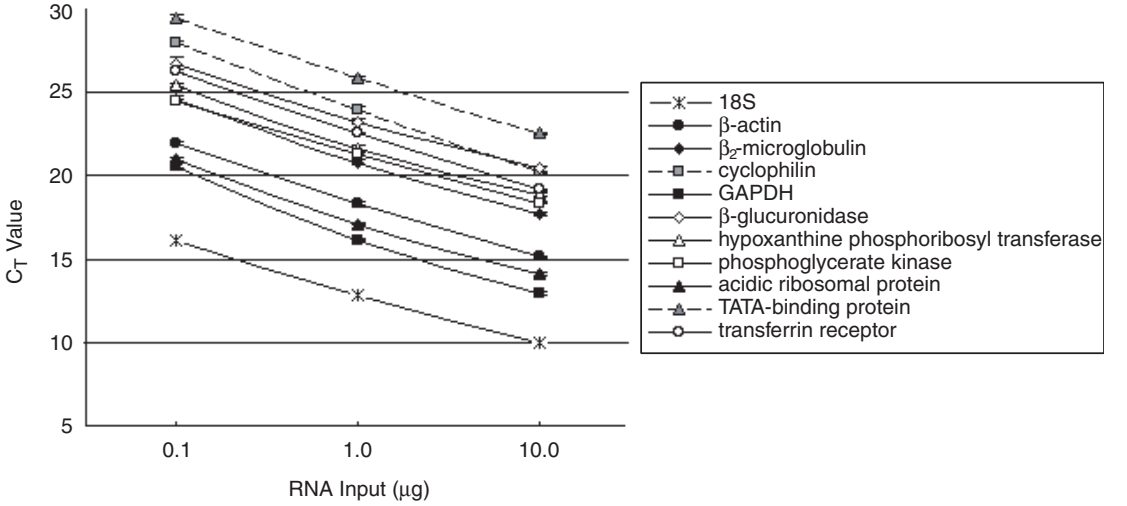


Figure 1 The expected ΔC_T values of 3.3 for each tenfold increase in the RNA input quantity are obtained for 11 different RNA transcripts converted to cDNA from different input quantities of total RNA.

Yield vs. Reaction Time

To achieve optimal conversion, Applied Biosystems recommends allowing reverse transcription to occur for 120 minutes at 37 °C.

Figures 2, 3, and 4 show C_T values plotted against reaction time in minutes for three different targets (18S, GAPDH, and β_2-microglobulin) and three input amounts of RNA (0.1, 1.0, and 10.0 µg in 100-µL reactions).

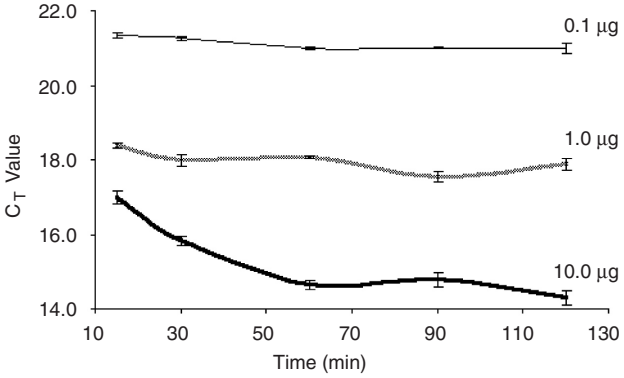


Figure 2 The rate of conversion of 18S RNA to cDNA reaches a maximum at 120 minutes with 10 µg of input RNA and at 30 minutes or less with 0.1 to 1.0 µg of input RNA (based on 100-µL reactions).

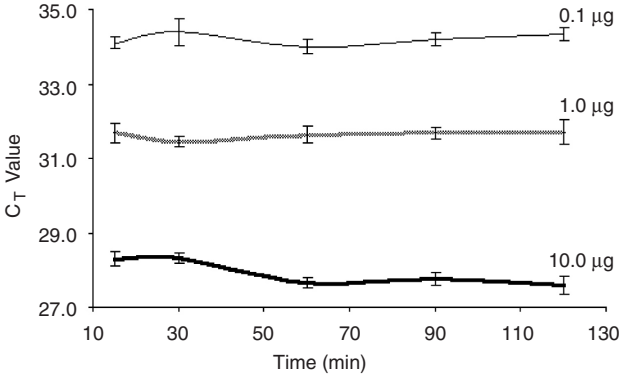


Figure 3 The rate of conversion of GAPDH RNA to cDNA reaches a maximum at 60 minutes or less at all RNA input levels (based on 100-µL reactions).

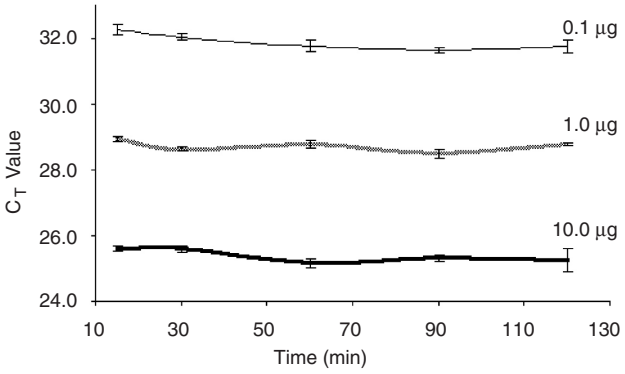


Figure 4 The rate of conversion of β_2-microglobulin RNA to cDNA reaches a maximum at 60 minutes or less at all RNA input levels (based on 100-µL reactions).

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