



Cells-to-C_T[™] 1-Step TaqMan[®] Kit

One-step RT-PCR in cell culture lysates

Buy Now Buy Now Buy Now

Catalog Number A25605, A25603, A25602

Publication Number MAN0010650

Revision A.0

Learn More



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About this guide

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

Revision history

Revision	Date	Description
A.0	June 2014	New document.



Product information

Product description

The Cells-to- $C_T^{\text{\tiny TM}}$ 1-Step TaqMan® Kit is designed for one-step reverse transcription and real-time PCR (real-time RT-PCR) in cell-culture lysates with TaqMan® PCR technology. The kit includes reagents for cell lysis, optional DNase treatment, reverse transcriptase, and real-time PCR in pre-mixed formulations that minimize pipetting steps. TaqMan® Gene Expression Assays for the target of interest are provided by the user. The Cells-to- $C_T^{\text{\tiny TM}}$ 1-Step TaqMan® Kit enables analysis of large numbers of samples with minimal risk of cross-contamination.

To provide a positive control for real-time RT-PCR and an indicator for the presence of RT-PCR inhibitors, RT-PCR targeting Xeno^{$^{\text{TM}}$} RNA Control from the TaqMan^{$^{\text{RNA}}$} Cellsto- $C_T^{^{\text{TM}}}$ Control Kit can be performed. See "Perform 1-step RT-PCR with Xeno RNA Control" on page 15.

Kit contents and storage

Table 1 Cells-to- C_T^{TM} 1-Step TaqMan[®] Kit

	Buy Now	Buy Now	Buy Now	
Component	Cat. no. A25605 (20 rxns) ^[1]	Cat. no. A25603 (100 rxns) ^[1]	Cat. no. A25602 (400 rxns) ^[1]	Storage
Lysis Solution	2.2 mL	5.5 mL	22 mL	2°C to 8°C ^[2]
DNase I	22 µL	55 µL	220 µL	-25°C to -15°C ^[3]
Stop Solution	200 μL	500 μL	2 × 1.1 mL	-25°C to -15°C ^[3]
TaqMan® 1-Step qRT-PCR Mix	250 μL	2.5 mL	10 mL	−25°C to −15°C

^{[1] 50-}µL lysis reactions and 20-µL RT-PCR reactions

Required materials not provided with the kit

Unless otherwise specified, all materials are available from Life Technologies (www.lifetechnologies.com). MLS: Fisher Scientific (www.fisherscientific.com) or other major laboratory supplier.

^[2] Reported as "4°C" on the component label.

^[3] Reported as "-20°C" on the component label.

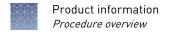
İtem	Source
Real-time PCR instrument: • Applied Biosystems® 7500 Real-Time PCR System • Applied Biosystems® 7500 Fast Real-Time PCR System • Applied Biosystems® 7900HT Fast Real-Time PCR System • StepOne™ Real-Time PCR System • StepOnePlus™ Real-Time PCR System • ViiA™ 7 Real-Time PCR System • QuantStudio™ Flex Systems	Contact your local or regional sales representative.
Tubes, plates, seals, and other accessories required by the instrument	Visit www.lifetechnologies.com, and select Life Sciences ➤ PCR ➤ PCR Tubes, Plates & Accessories
Laboratory mixer (Vortex mixer or equivalent)	MLS
(If needed) Benchtop centrifuge, for washing suspension cells	MLS
Microcentrifuge	MLS
Adjustable micropipettors, 10 μL to 1000 μL	MLS
Aerosol-resistant pipette tips	MLS
Nuclease-Free Water	Cat. no. AM9938
10X PBS ^[1]	Cat. nos. AM9624, AM9625
TaqMan [®] Gene Expression Assay for the target of interest	Visit www.lifetechnologies.com
(Optional) TaqMan® Cells-to-C _T ™ Control Kit	Cat. no. 4386995

^[1] Dilute to 1X with Nuclease-Free Water before use.

Procedure overview

First, $10 \text{ to } 10^5 \text{ cultured cells}$ are washed with phosphate-buffered saline (PBS), then mixed with Lysis Solution and incubated at room temperature for 5 minutes. Cells are lysed during this incubation and RNA is released into the Lysis Solution, which contains reagents to inactivate endogenous RNases. If DNase I is added to the Lysis Solution (optional), genomic DNA is also degraded at this step.

Next, Stop Solution is mixed into the lysate to inactivate the lysis reagents so that they will not inhibit the reverse transcription or PCR reactions. The resulting lysate is then used in one-step real-time RT-PCR with a TaqMan[®] Gene Expression Assay for the target of interest.



Workflow

Prepare cells for lysis

Adherent cells in 96- or 384-well plates: rinse with 1X PBS, then aspirate PBS.

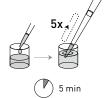
Cells grown in other vessels (adherent or suspension): wash cells in 1X PBS, resuspend in 1X PBS at 10 to 10^5 cells per 5 μ L, and transfer 5 μ L to a 96-well plate.





Prepare the Cells-to-C_T™ lysate

Add 50 μL of Lysis Solution to each well, pipette 5 times, and incubate at room temp. for 5 min.



 \blacksquare

Add 5 μ L of Stop Solution, pipette 5 times, and incubate at room temp for 2 min.



(1) 2 mi



Combine TaqMan® 1-Step qRT-PCR Mix with TaqMan® Gene Expression Assay and lysate, set up the instrument, then run the reaction.



Methods

Important procedural guidelines

Cells-to-C_T[™] lysate preparation

- The lysis procedure is optimized for lysis of 10 to 10⁵ cells per reaction.
- Do not vortex Stop Solution.
- Lysis Solution and Stop Solution must be at room temperature before starting the lysis procedure.
- We recommend storing Stop Solution in several tubes or plate wells (96-well format), for faster thawing and to reduce the number of freeze-thaw cycles.
- Avoid creating bubbles in the lysis procedure.
 For example, when mixing in Lysis Solution or Stop Solution, set the pipettor to ~35 μL to pipet the ~50-μL reactions.
- DNase treatment is recommended if primers and/or probes do not span an exon junction.
- The minimum incubation time after addition of Stop Solution is 2 minutes. The incubation time can be extended to accommodate handling a large number of samples, but do not allow the reactions to remain at room temperature longer than 20 minutes after addition of Stop Solution.

RT-PCR setup

• Cell type is the most significant factor in determining the optimal input lysate amount for the RT-PCR reaction.

We recommend performing an optimization experiment when using the Cells-to- $C_T^{\text{\tiny TM}}$ 1-Step TaqMan[®] Kit for the first time with a cell type.

- Vary the number of cells in the lysis reaction between 10 and 10⁵ cells.
- Vary the volume of the lysate in the RT-PCR between 2% and 10% of the total RT-PCR reaction volume.
- The volume of the lysate should be between 2% and 10% of the total RT-PCR reaction volume; for most cell types, good results are seen with a lysate volume of 5%.

Do not exceed:

- 1600 cell equivalents for 20-μL reactions.
- 4000 cell equivalents for 50-μL reactions.
- Do not pipette less than 1 µL of lysate.

If less than 1 μ L of lysate is required, dilute a portion of the lysate with Nuclease-Free Water, to pipette a larger volume, and reduce the volume of water in the RT-PCR reaction accordingly.

Include a no-template control (NTC) reaction: Use Nuclease-Free Water instead
of lysate in the RT-PCR.

This control tests for DNA contamination in the reagents.

- Include an appropriate number of PCR replicates or biological replicates, as required by your experimental needs.
- Follow "Good laboratory practices for PCR and RT-PCR" on page 15.

Before each use of the kit

- Chill 1X PBS on ice, sufficient for $50 \mu L$ per 10^5 cells.
- Thaw Stop Solution, with gentle mixing (*do not vortex*). Ensure that Stop Solution is at room temperature before use.
- (Optional) If DNase treatment is desired, prepare DNase/Lysis Solution master mix for the number of reactions required plus 10% overage. Prepare the master mix just before use.

Component	Volume per reaction
Lysis Solution (room temperature)	49.5 μL
DNase	0.5 μL

Prepare cells for lysis

Prepare adherent or suspension cells for lysis.

Cell type	To prepare cells for lysis
Adherent cells grown in 96- or 384- well plates	Use cells that have been cultured until they are fully adherent to the plate, to avoid losing cells during the wash.
	 Aspirate culture medium from the wells and rinse with 50 μL of cold 1X PBS.
	Aspirate as much PBS as possible without disturbing the cells and proceed to "Prepare the Cells-to-CT lysate" on page 11.
Cells grown in other vessels, including adherent and suspension	Detach adherent cells from the culture vessel, using a common subculturing technique such as trypsin.
cells	If trypsin is used, be sure to inactivate it before proceeding.
	2. Count the cells, then gently centrifuge to pellet the cells.
	 Discard the growth medium and resuspend the pellet in ~50 μL of chilled 1X PBS per 10⁵ cells.
	 Gently pellet the cells, and aspirate as much PBS as possible without disturbing the pellet.
	5. Resuspend in 5 µL of cold 1X PBS per 10 to 10 ⁵ cells, and pipette up and down to thoroughly resuspend the cells.
	6. Distribute 5 μL of cells to the desired number of wells of a 96-well PCR plate and proceed to "Prepare the Cells-to-CT lysate" on page 11.

Prepare the Cells-to-C_T[™] lysate

- 1. Add $50 \mu L$ of room-temperature Lysis Solution or DNase/Lysis Solution to the prepared cells, and pipette up and down 5 times to mix well.
- **2.** Incubate at room temperature for 5 minutes.
- 3. Add 5 μ L of room-temperature Stop Solution and pipette up and down 5 times.
 - If you are using a multi-channel pipette, Stop Solution can be added directly to the lysis reaction.
 - If you are using a repeater pipette, Stop Solution can be added to the side wall of the well, but it is important to rinse the wall when pipetting up and down, to ensure thorough mixing of all of the Stop Solution with the lysis reaction.
- **4.** Incubate at room temperature for 2 minutes.
- 5. Place the lysates on ice, and proceed to RT-PCR.

STOPPING POINT Lysates can be stored on ice for up to 2 hours or at or below -20°C for up to 5 months.

Perform 1-step RT-PCR

- 1. Thaw all reagents, including previously frozen Cells-to- $C_T^{^{\text{TM}}}$ lysates, on ice. TaqMan® 1-Step qRT-PCR Mix is very viscous; invert the tube 2–3 times to ensure that it is thawed completely and mixed thoroughly.
- **2.** On ice, prepare RT-PCR Master Mix for the number of reactions required plus 10% overage.

Adjust the final volume of RT-PCR Master Mix for the amount of lysate in each reaction.

Table 2 RT-PCR Master Mix (for 20-µL reactions)

Component	Volume per 20-µL reaction ^[1]
TaqMan® 1-Step qRT-PCR Mix	5 μL
TaqMan [®] Gene Expression Assay, 20X	1 μL
Nuclease-Free Water	To 19 μL (for 1 μL of lysate) To 18 μL (for 2 μL of lysate)

 $^{^{[1]}}$ Component volumes can be scaled for RT-PCR reaction volumes between 10 μL and 50 μL

- 3. On ice, add the appropriate volume (18–19 μ L) of RT-PCR Master Mix to each sample or control well of an optical reaction plate.
- **4.** Add the appropriate volume (1–2 μ L) of lysate or Nuclease-Free Water (for the NTC) to each well (20 μ L total).

- **5.** Seal the plate with an optical adhesive cover, vortex the plate for 5–10 seconds, then briefly centrifuge the plate.
 - TaqMan® 1-Step qRT-PCR Mix is more viscous than most master mixes. Ensure that the contents of the plate are mixed well and then briefly centrifuge to remove air bubbles that may have formed in the wells and to bring the contents to the bottom.
- **6.** Set up the real-time PCR instrument as indicated in the following table, then load and run the reactions.

Table 3 Standard cycling conditions

Step	No. of cycles	Temp.	Time
Reverse transcription	1	50°C	5 min
RT inactivation/initial denaturation	1	95°C	20 sec
Amplification	40	95°C	15 sec
		60°C	1 min

Table 4 Fast cycling conditions

Step	No. of cycles	Temp.	Time
Reverse transcription	1	50°C	5 min
RT inactivation/initial denaturation	1	95°C	20 sec
Amplification	40	95°C	3 sec
		60°C	30 sec



Troubleshooting

For general real-time RT-PCR troubleshooting, refer to the appropriate guide for your instrument, or visit www.lifetechnologies.com and select Life Sciences

▶ PCR ▶ Real-Time PCR (qPCR) ▶ qPCR Education.

Cells-to-C_T[™] troubleshooting

Observation	Possible cause	Recommended action
No signal or unexpected signal, due to RT-PCR inhibition The presence of RT-PCR inhibitors in the lysate has been	Components in the Lysis Solution are not fully inactivated by Stop Solution.	Add Stop Solution directly to the lysate: touch the lysate with the opening of the pipet tip when adding Stop Solution to ensure that the entire 5 μ L of Stop Solution is added to each sample.
confirmed after following "Perform 1-step RT-PCR with Xeno RNA Control" on		Mix Stop Solution with the lysate by pipetting up and down 5 times.
page 15.	Too many cells were used in the lysis reaction, therefore RNase in the sample was not	Repeat the entire procedure (lysis and RT-PCR) with fewer cells (for example, 5- to 10-fold fewer cells).
	completely inactivated and/or cellular components or debris inhibited the RT-PCR.	Perform an optimization experiment to determine the optimal number of cells for your cell type (see "RT-PCR setup" on page 9).
	Too much PBS (>5 μL) was left on the cells, diluting the Lysis	Remove as much PBS as possible before adding Lysis Solution to the cells.
	Solution, therefore RNase in the sample was not completely inactivated.	If you split the cells after the PBS wash, resuspend cells in $\leq 5~\mu L$ of PBS for each sample of 10 to 10^5 cells.

RNA was degraded before starting the procedure. Lysis Solution was not at room temperature, therefore cell lysis was not efficient. Lysates sat too long at room temperature before the RT-PCR reaction.	To avoid RNA degradation, keep cells in PBS on ice before starting the lysis procedure. Take cells off ice just before adding Lysis Solution. Ensure that Lysis Solution is at room temperature before starting the lysis procedure. Do not allow lysates to sit longer than 20 minutes at room temperature once the Stop Solution has been added. If the RT-PCR reactions cannot be set up within this time: • Store the lysates on ice for up to 2 hours after lysis.
temperature, therefore cell lysis was not efficient. Lysates sat too long at room temperature before the RT-	temperature before starting the lysis procedure. Do not allow lysates to sit longer than 20 minutes at room temperature once the Stop Solution has been added. If the RT-PCR reactions cannot be set up within this time: • Store the lysates on ice for up to 2 hours after lysis.
temperature before the RT-	20 minutes at room temperature once the Stop Solution has been added. If the RT-PCR reactions cannot be set up within this time: • Store the lysates on ice for up to 2 hours after lysis.
	after lysis.
	- Facasa
	• Freeze the lysates at or below –20°C.
The sample does not contain the target RNA.	Before concluding that the sample does not contain the RNA of interest:
	 Follow "Perform 1-step RT-PCR with Xeno RNA Control" on page 15 to determine whether the lysate contains RT-PCR inhibitors, but also perform parallel realtime RT-PCR reactions with the TaqMan® Gene Expression Assay for the target of interest. If PCR product is detected in the Xeno™ RNA Control reaction but not in the PCR for the RNA of interest, then it is possible that the RNA of interest is not expressed in the cells or is undetectable with this procedure.
	 Perform RT-PCR using purified RNA from the same cell culture source, to confirm that the PCR reagents and equipment work for the target of interest.
DNA contamination of	Repeat the procedure with fresh reagents.
reagents, pipettors, or benchtops.	Follow "Good laboratory practices for PCR and RT-PCR" on page 15.
The RT-PCR reaction was not thoroughly mixed.	After assembly of the RT-PCR reactions in the plate, be sure to vortex the reaction plate briefly (5–10 seconds) before centrifuging the plate.
	DNA contamination of reagents, pipettors, or benchtops. The RT-PCR reaction was not

A

Perform 1-step RT-PCR with Xeno™ RNA Control

Xeno[™] RNA Control is a synthetic RNA transcript that lacks homology to current annotated biological sequences.

In this experiment, $Xeno^{^{\intercal}}RNA$ Control and the $Xeno^{^{\intercal}}RNA$ Control TaqMan[®] Gene Expression Assay from the TaqMan[®] Cells-to- $C_T^{^{\intercal}}$ Control Kit are used for RT-PCR in the presence and absence of lysate. If signal for $Xeno^{^{\intercal}}RNA$ Control is detected only in reactions that do not contain lysate, or if reactions with lysate display a significantly higher C_T (lower signal) than reactions without lysate, this indicates that RT-PCR inhibitors are present in the lysate.

The TaqMan® Cells-to- $C_T^{\text{\tiny TM}}$ Control Kit also includes a TaqMan® Gene Expression Assay for the endogenous control gene β -Actin (ACTB) providing an endogenous control for sample normalization (see

"Don't Bother Quantitating RNA for Gene Expression Analysis" at www.lifetechnologies.com). Refer to the product information sheet for the TaqMan[®] Cells-to- C_T^{TM} Control Kit (Pub. no. 4387227) for other applications of the kit.

1. Follow the main procedure, but prepare an RT-PCR Master Mix that includes Xeno[™] RNA Control and Xeno[™] RNA Control TaqMan[®] Gene Expression Assay instead of the gene-specific assay, for the number of reactions plus 10% overage. Prepare the master mix to accommodate the same volume of lysate that is used in RT-PCRs for the experimental samples.

Table 5 RT-PCR Master Mix with Xeno[™] RNA Control (for 20-µL reactions)

Component	Volume per 20-µL reaction
TaqMan® 1-Step qRT-PCR Mix	5 μL
20X Xeno [™] RNA Control TaqMan [®] Gene Expression Assay	1 μL
Xeno [™] RNA Control	0.036 μL ^[1]
Nuclease-Free Water	To 19 μL (for 1 μL of lysate) To 18 μL (for 2 μL of lysate)

^[1] Use 3.6 µL of Xeno[™] RNA Control per 100 20-µL reactions. Dilute Xeno[™] RNA Control in Nuclease-Free Water for fewer reactions.

- 2. Add the appropriate volume (1–2 μ L) of lysate or Nuclease-Free Water (for the NTC) to each well.
- **3.** Continue the RT-PCR procedure as for the experimental samples.

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 (not previously worn while handling amplified products or during sample preparation).
- Change gloves whenever you suspect that they are contaminated.

Appendix A Troubleshooting Good laboratory practices for PCR and RT-PCR

- 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 DNAZap[™] Solutions (Cat. no. AM9890).



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, and 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. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Safety equipment also may 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

Obtaining SDSs

Safety Data Sheets (SDSs) are available from www.lifetechnologies.com/support.

Note: For SDSs of chemicals from third-party manufacturers, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

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.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

