invitrogen

TaqMan™ Fast Advanced Cells-to-C_T™ Kit

USER GUIDE

Catalog Numbers A35374, A35377, A35378

Publication Number MAN0017754

Revision B.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: Pub. No. MAN0017754

Revision	Date	Description
B.0	26 July 2022	A product name was updated in the perform qPCR task.
A.0	14 June 2018	Initial release.

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

Contents

1 Product information

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The Invitrogen™ TaqMan™ Fast Advanced Cells-to-C_T™ Kit includes reagents and enzyme mixtures for reverse transcription (RT) and real-time PCR directly from cultured cell lysates, without isolating RNA.

Cells-to-C_T™ technology enables reverse transcription of lysates from 10–10^5 cultured cells without isolating or purifying RNA. Real-time PCR analysis is carried out directly afterwards. Eliminating the RNA isolation step substantially expedites and simplifies gene expression analysis of cultured cells. Cells-to-C_T™ lysates exhibit sensitivity and specificity similar to that from purified RNA in real-time RT-PCR. The lysis procedure simultaneously prepares cell lysates for RT-PCR and removes genomic DNA, in under ten minutes. The lysis step is simple to automate with robotic platforms for high-throughput processing of 96- or 384-well plates, because it takes place entirely at room temperature. The procedure is also economical as there are only a few pipetting steps and, with cells grown in 96- or 384-well plates, no sample transfers.

Contents and storage

Component	Cat. No. A35374 (40 rxn)	Cat. No. A35377 (100 rx)	Cat. No. A35377 (400 rx)	Storage
Stop Solution	200 µL	500 µL	2 x 1.0 mL	–20°C
DNase I	22 µL	55 µL	220 µL	
20X RT Fast Advanced Enzyme Mix	110 µL	275 µL	1.1 mL	
2X Fast Advanced RT Buffer	2.2 mL	5.5 mL	22 mL	–20°C [1]
Lysis Solution	2.2 mL	5.5 mL	22 mL	4°C
TaqMan™ Fast Advanced Master Mix	1.0 mL	5.0 mL	4 x 5.0 mL	

[1] After the RT Buffer is thawed for the first time, it can be stored at 4°C.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

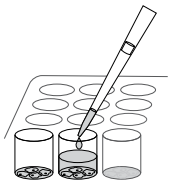
Item	Source
Instrument	
For reverse transcription, one of the following or equivalent:	
Veriti™ 96-Well Thermal Cycler	4375786
For real-time PCR, one of the following:	
QuantStudio™ 3 and 5 Real-Time PCR Systems	A31668, A31671
QuantStudio™ 6 and 7 Flex	4485697, 4485698
QuantStudio™ 12K Flex	4471050
Equipment	
Vortex mixer	**MLS**
Microcentrifuge	**MLS**
Pipets	**MLS**
Consumables	
Nuclease free pipette tips	**MLS**
Nuclease free microcentrifuge tubes	**MLS**
U-bottom 96-well plates (for cells not cultured in 96- or 384-well plates)	**MLS**
Real-time PCR tubes or multiwell plates appropriate for your instrument	**MLS**
Reagents	
RT-PCR grade water	**MLS**
PBS (1X), pH 7.4	AM9624
(Optional) TaqMan™ Cells-to-C_T™ Control Kit	4386995

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 µL, and transfer 5 µL to a 96-well plate.



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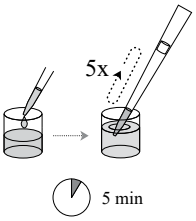
Prepare the Cells-to-C_T™ lysate

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



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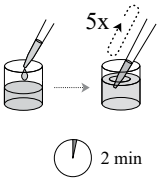
Add 5 µL of Stop Solution, pipette 5 times or use an orbital shaker, and incubate at room temp for 2 min.



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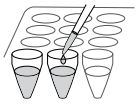
Perform reverse transcription

Combine the RT Master Mix with the lysate, set up the instrument, then run the reaction.



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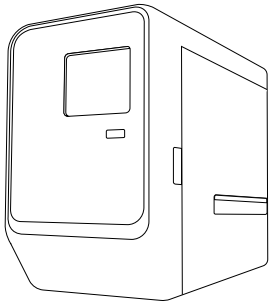
Perform qPCR

Combine the PCR Cocktail with the cDNA, set up the instrument, then run the reaction.



2 Methods

Procedural guidelines

- Using too many cells per lysis reaction can result in incomplete lysis and or inhibition of RT-PCR. The maximum number of cells that can be used for this procedure varies according to cell type, and is generally 10^5 cells. To determine the maximum number of cells per reaction, refer to *Cell input optimization for TaqMan™ Fast Advanced Cells-to-C_T™ Kit User Bulletin* (Pub. No. MAN0017932).
- Use fresh cultured cells. When using frozen cultured cells, ensure that the cells were washed in cold PBS before freezing. Start the procedure by thawing the cells on ice and proceeding from "Prepare the Cells-to-CT lysate" on page 9.
- To reduce wasted reagent, dispense Stop Solution using a multichannel pipet from a set of strip tubes or a 96-well plate instead of a reagent reservoir.
- To avoid bubble formation when mixing, set the pipet to less than the reaction volume and expel the solution without emptying the pipet tip completely.
- Unless otherwise stated, room temperature is 20–25°C.
- Minus-RT controls contain all the RT reaction components except the 20X RT Enzyme Mix (substitute water). Minus-RT controls show that the template for the PCR was cDNA, and not genomic DNA.
- No-template controls (NTC) contain all the PCR components except the cell lysate (substitute water). If the no-template control yields a fluorescent signal, the RT or PCR reagents can be contaminated with DNA, for example, PCR product from previous reactions.

Before you begin

- Thaw the Stop Solution and mix thoroughly by inverting or flicking several times, then place on ice.

  IMPORTANT! Do not vortex.

- Chill 1X PBS to 4°C.

- *(Optional)* Add DNase I (1:100) to Lysis Solution, to remove genomic DNA during cell lysis, according to the following table.

Component	Volume		
	per reaction	96 reactions[1]	384 reactions
Lysis Solution	49.5 µL	5.23 mL	20.91 mL
DNase I	0.5 µL	52.8 µL	211 µL
Total	**50 µL**	**5.28 mL**	**21.12 mL**

[1] Includes 10% overage

- *(Optional)* To include an exogenous control using the TaqMan™ Cells-to-C_T™ Control Kit, add 1 µL of Xeno™ RNA Control per 5 µL of Stop Solution.

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. **1.** Ensure each well contains 10–10^5 cells. **2.** Aspirate and discard the culture medium from each well. **3.** Add 50 µL of 4°C 1X PBS to each well. **4.** Aspirate and discard the PBS from each well. **Note:** Remove as much PBS as possible, without disturbing the cells.
Cells grown in other vessels, including adherent and suspension cells	**1.** Detach adherent cells from the culture vessel, using a common subculturing technique such as trypsin. If trypsin is used, be sure to inactivate it before proceeding. **2.** Count the cells, then gently centrifuge to collect the contents at the bottom. **3.** Aspirate and discard the medium, then place the cells on ice. **4.** Wash the cells by resuspending them in 0.5 mL of 4°C PBS per 10^6 cells, then gently centrifuge to collect the contents at the bottom. **5.** Aspirate and discard as much of the PBS as possible without disturbing the pellet, then place the cells on ice. **6.** Resuspend the cells in fresh, 4°C PBS so that 5 µL contains the desired number of cells for one lysis reaction (10–10^5 cells/reaction). **7.** Split the cell suspension into individual lysis reactions (5 µL per reaction) in a U-bottom multiwell plate or individual microcentrifuge tubes, then place on ice.

Prepare the Cells-to-CT lysate

1. Add 50 µL of Lysis Solution to each sample, then mix the lysis reaction by pipetting up and down 5 times or by gentle shaking on an orbital shaker.

   Note: To remove genomic DNA add DNase I to Lysis Solution, see “Before you begin” on page 8.

2. Incubate the lysis reaction for 5 minutes at room temperature.

3. Add Stop Solution to each lysis reaction according to the following table.

Option	Volume to add
Stop Solution	5 µL
Stop Solution with Xeno™ RNA Control	6 µL

Note: Touch the surface of the lysate with the opening of the pipet tip to ensure that all the Stop Solution is added to the lysate.

4. Mix the lysis reaction by pipetting up and down five times or by gentle shaking on an orbital shaker.

IMPORTANT! Thoroughly mix the Stop Solution into the lysate.

5. Incubate for 2 minutes at room temperature.

STOPPING POINT Lysates can be stored on ice for ≤2 hours, or at –20°C to –80°C for ≤5 months.

Perform reverse transcription (RT)

1. In a nuclease-free microcentrifuge tube on ice, prepare an RT Master Mix for the number of reactions required plus 10% overage, according to the following table.

   Up to 45% of the RT reaction volume (22.5 µL) can be Cells-to-C_T™ lysate. Adjust the volume of Nuclease-free Water accordingly.

Component	1 reaction	96 reactions[1]	384 reactions[1]
2X Fast Advanced RT Buffer	25 µL	2.64 mL	10.56 mL
20X Fast Advanced RT Enzyme Mix[2]	2.5 µL	264 µL	1.056 mL
Nuclease-free water	12.5 µL	1.32 mL	5.28 mL
Total	**40 µL**	**4.22 mL**	**16.9 mL**

[1] Volumes include 10% overage.
[2] For the minus-RT control, use Nuclease-free water instead of 20X Fast Advanced RT Enzyme Mix.

Note: If a 50-µL RT reaction will not provide sufficient material for all the planned PCRs, the RT reaction can be scaled up proportionally.

2. Mix the RT Master Mix gently but thoroughly, then briefly centrifuge and place on ice.
3. Distribute RT Master Mix to nuclease-free PCR tubes or wells of a multiwell plate.
4. Add sample lysate to each aliquot of RT Master Mix for a final 50-µL reaction volume.

5. Mix reactions gently, then centrifuge briefly to collect the contents at the bottom of the reaction container.

STOPPING POINT Assembled RT reactions can be stored at 4°C for up to 4 hours.

6. Set up the thermal cycler (or real-time PCR instrument) as indicated in the following table, then load and run the reactions.

Step	Stage	Cycles	Temperature	Time
Reverse transcription (hold)	1	1	37°C	30 minutes
RT inactivation (hold)	2	1	95°C	5 minutes
Hold	3	1	4°C	Indefinite

STOPPING POINT Completed RT reactions can be stored at –20°C.

Perform qPCR

1. In a nuclease-free microcentrifuge tube at room temperature, prepare the PCR Cocktail plus 10% overage according to the following table.

Component	10 µL PCR reaction	20 µL PCR reaction
TaqMan™ Fast Advanced Master Mix	5 µL	10 µL
TaqMan™ Gene Expression Assay	0.5 µL	1 µL
Nuclease-free water	2.5 µL	5 µL
Total	8 µL	**16 µL**

Note: Up to 45% of the PCR volume can be cDNA, we recommend 20%.

2. Distribute the PCR Cocktail into individual PCR tubes or wells of a real-time PCR plate at room temperature.

3. Add cDNA to the PCR Cocktail according to the following table.

Component	10 µL PCR reaction	20 µL PCR reaction
PCR Cocktail	8 µL	16 µL
cDNA (RT reaction)	2 µL	4 µL

4. Close the tubes or cover the plate, then mix gently.

5. Centrifuge briefly to remove bubbles and collect the contents at the bottom of the tubes/wells.

6. Set up the real-time PCR instrument as indicated in the following table, then load and run the reactions.

   Specify the fluorescent dye used in the TaqMan™ Gene Expression Assay for the experiment. The ACTB and Xeno™ RNA Gene Expression Assays in the TaqMan™ Cells-to-C_T™ Control Kit are labeled with FAM™ dye and a nonfluorescent quencher.

Step	Stage	Cycles	Temperature	Time
UDG activation	1	1	50°C	2 minutes
Enzyme activation (hold)	2	1	95°C	20 seconds
PCR	3	40	95°C	1 seconds
			60°C	20 seconds

IMPORTANT! TaqMan™ Fast Advanced Master Mix contains ROX™ passive reference dye.

A

Troubleshooting

Observation	Possible cause	Recommended action
No PCR product or unexpected PCR products	Problems with adding or mixing the Stop Solution	Components in the Lysis Solution can inhibit RT-PCR if they are not inactivated by the Stop Solution.
		Add the Stop Solution directly to the lysate. Touch the lysate with the opening of the pipette tip when adding the Stop Solution to ensure that the entire 5 µL or 6 µL of Stop Solution is added to each sample.
		Mix by pipetting up and down five times or by using an orbital shaker.
	RNA was degraded before starting the procedure	To avoid RNA degradation, keep cells in PBS on ice before starting the cell lysis procedure. Take cells off ice just before adding Lysis Solution.
	RNase in the sample was not inactivated: Too many cells were used in the lysis reaction.	If too many cells per sample are used in the procedure, the RNase in the sample cannot be inactivated and/or cellular components or debris could inhibit reverse transcription or PCR.
		Generally ≤10^5 cells can be used successfully in the Cells-to-C_T™ procedure, but if RT or PCR fails, try using 5-to 10-fold fewer cells.
		Perform a pilot experiment to determine the optimal number of cells for your cell type. Refer to *Cell input optimization for TaqMan™ Fast Advanced Cells-to-C_T™ Kit User Bulletin* (Pub. No. MAN0017932).
		Suboptimal primer design can become apparent at higher cell inputs.
	RNase in the sample was not inactivated: Too much PBS was left on the cells, diluting the Lysis Solution	If >5 µL of PBS remains in the samples when the Lysis Solution is added, the Lysis Solution is too diluted to inactivate cellular RNases. Remove as much PBS as possible before adding Lysis Solution to the cells, or if you split the cells after the PBS wash, resuspend cells in ≤5 µL PBS for each sample of 10–10^5 cells.

Observation	Possible cause	Recommended action
No PCR product or unexpected PCR products *(continued)*	Lysates sat too long before going into RT	Do not allow lysates to sit longer than 20 minutes at room temperature after the Stop Solution has been added. Either freeze the lysates at –20°C or –80°C, or start the RT reactions. Alternatively, lysates can be safely stored on ice for up to 2 hours after lysis.
	The sample does not contain the target RNA	Negative results are often difficult to confirm as valid. Run the following experiments before concluding that the sample does not contain the RNA of interest. • Verify that the procedure is working by including Xeno™ RNA Control (from the TaqMan™ Cells-to-C_T™ Control Kit) in the sample, see "Prepare the Cells-to-CT lysate" on page 9. Then use the Xeno™ RNA Control TaqMan™ Assays to amplify a Xeno RNA target, see "Perform qPCR" on page 11. If product is generated in the Xeno RNA amplification, but no product is seen in the PCR for the gene of interest, then it is possible that the RNA of interest is not expressed in the cells and/or is undetectable with this procedure. • For experiments with samples consisting of <100 cells per lysis, verify that each sample contains cells. Check that samples contained cells with intact RNA by real-time RT-PCR with TaqMan™ Assays for a highly expressed endogenous control, such as β-actin. The ACTB primers included in the TaqMan™ Cells-to-C_T™ Control Kit) are designed for this purpose. • Check that the PCR for your target works with your PCR primers, reagents, and equipment by using cDNA generated from purified RNA from the same source (or a similar one) in PCR. If the amplification does not give good results using cDNA from purified RNA, it will not work with Cells-to-C_T™ lysate.
	Inhibitors in the RT reaction or PCR	Check for the presence of RT or PCR inhibitors in the Cells-to-C_T™ lysate by purifying the RNA from the lysate using a standard method, then compare RT-PCR results from the purified RNA to that from the lysate.

Observation	Possible cause	Recommended action
RT-PCR products in the negative control reactions	PCR products in the no-template PCR control	Decontaminate pipets and benchtops, then replace contaminated reagents. Careful laboratory practices are essential to avoid contaminating reactions with PCR products. Keep concentrated DNA solutions (for example, PCR products, plasmid preps) away from the area where PCRs are assembled. Routinely clean the lab bench and pipettes with DNA*Zap*™ PCR DNA Degradation Solutions (Cat. No. AM9890) or another DNA decontamination product. Use barrier tips to pipet PCR reagents, and store completed PCRs in a different location from the PCR reagents.
		Include a no-template negative control reaction with experimental PCRs.
	PCR products in the minus-RT control	• Ensure DNase I is thoroughly mixed into the Lysis Solution for efficient removal of genomic DNA from samples. • Use TaqMan™ Assays that are designed to span an exon-exon boundary. Amplicons from genomic DNA would therefore be too long for efficient PCR amplification. • Use fewer cells per lysis reaction. • Lyse cells using Lysis Solution that is at room temperature, and ensure that lysis reactions occur at room temperature (19–25°C).
		If PCR products are still seen in minus-RT control reactions, try the following two suggestions in the order shown: • Increase the lysis reaction incubation time to 8 minutes. • Use Lysis Solution that has been warmed to 25°C for cell lysis.

B

Supplemental information

Applications

The TaqMan™ Fast Advanced Cells-to-C_T™ Kit can be used in any real-time RT-PCR application to analyze mRNA from cultured cells. The kit is well suited for large experiments, such as real-time RT-PCR analysis of gene expression in differentially treated cell cultures or RNAi screening experiments using siRNA to modulate gene expression. Other applications include screening a library of compounds for their effects on mRNA expression, following the regulation of mRNA as cells are treated with increasing concentrations of a particular chemical, or evaluating the expression of mRNA in time course experiments.

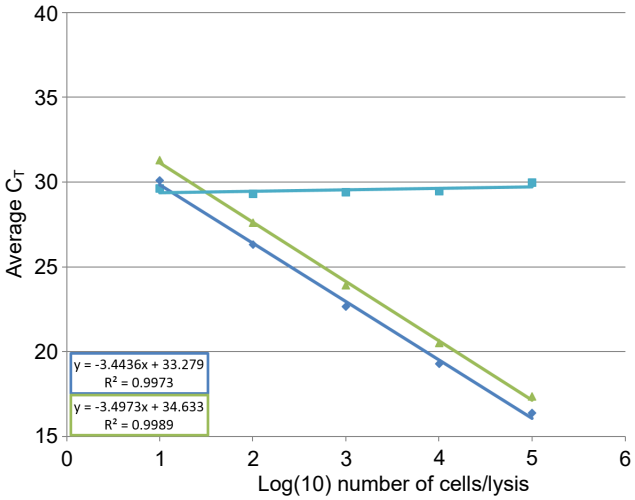


Figure 1 Real-time RT-PCR using the TaqMan™ Fast Advanced Cells-to-C_T™ Kit

A dilution series of 10^1 to 10^5 HeLa cells was processed in triplicate with the TaqMan™ Fast Advanced Cells-to-C_T™ Kit. The endogenous control gene β-actin (dark blue diamond) and the TKT gene (green triangle), along with Xeno control (light blue square), were amplified from the cDNA in duplicate 10 µL reactions. The standard curve shows the threshold cycle (C_t) compared to the input number of cells. Amplification was linear over a cell input range of 10^1 to 10^5 cells per lysis. The Xeno control C_ts remain constant over the entire cell input range, indicating the lysates do not inhibit RT-qPCR.

Cell types compatible with the Cells-to-C_T™ technology

Cell line	Growth	Source species	Source tissue
A549	Adherent	*H. sapiens*	Lung Carcinoma
BJ	Adherent	*H. sapiens*	Foreskin fibroblast
CHO-K1	Adherent	*C. griseus (hamster)*	Ovary
COS-7	Adherent	*C. griseus (hamster)*	Kidney
DU-145	Adherent	*H. sapiens*	Prostate Carcinoma
HEK-293	Adherent	*H. sapiens*	Kidney
HeLa	Adherent	*H. sapiens*	Cervical Adenocarcinoma
HepG2	Adherent	*H. sapiens*	Liver Carcinoma
Huh-7	Adherent	*H. sapiens*	Liver Carcinoma
Jurkat	Suspension	*H. sapiens*	Acute T-Cell Leukemia
K-562	Suspension	*H. sapiens*	Chronic Myelogenous Leukemia
ME-180	Adherent	*H. sapiens*	Cervical Epidermoid Carcinoma
NCI-H460	Adherent	*H. sapiens*	Large Cell Lung Cancer
Neuro 2A	Adherent	*M. musculus (mouse)*	Brain blastoma
NIH/3T3	Adherent	*M. musculus (mouse)*	Embryonic Fibroblast
PC-12	Adherent	*R. norvegicus (rat)*	Adrenal Pheochromocytoma
Primary Hepatocytes	Adherent	*H. sapiens*	Liver
PT-K75	Adherent	*S. scrofa (pig)*	Nasal Turbinate Mucosa
Raji	Suspension	*H. sapiens*	B Lymphocyte
SK-N-AS	Adherent	*H. sapiens*	Brain Neuroblast
SK-N-SH	Adherent	*H. sapiens*	Brain Fibroblast
U-87 MG	Adherent	*H. sapiens*	Brain Glioblastoma
U-2 OS	Adherent	*H. sapiens*	Bone osteosarcoma

C

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, and so on). To obtain SDSs, visit **thermofisher.com/support**.

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:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/publications/i/item/9789240011311

Documentation and support

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  - 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 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**.