

TaqMan™ Cells-to-CT™ Express Kit

USER GUIDE

Catalog Numbers A57985, A57986, A57987, A57988, A57989

Publication Number MAN0029716

Revision B



Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania
For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0029716 B (English)

Revision	Date	Description
B	15 November 2024	Reagent volumes for kit contents were updated.
A.0	28 July 2023	New document for the TaqMan™ Cells-to-CT™ Express Kit.

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

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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](https://www.thermofisher.com/support).

Product description

The Invitrogen™ TaqMan™ Cells-to-CT™ Express Kit includes reagents and enzyme mixtures to perform reverse transcription (RT) and qPCR directly from cultured cell lysates, without isolating RNA.

With Express technology, Cells-to-CT™ has been made even simpler with only one step for cell lysis, and no need for the addition of a Stop Solution. In this workflow, 10^3 – 10^5 cultured cells (adherent or suspension) are lysed with the Express Lysis Solution. The optional inclusion of Invitrogen™ ezDNase™ to the Express Lysis Solution enables removal of genomic DNA during cell lysis. Following a five minute incubation at room temperature, the lysate is used directly for reverse transcription using Invitrogen™ SuperScript™ IV VILO™ Master Mix. Once the RT reaction is complete, qPCR analysis is carried out using TaqMan™ Fast Advanced Master Mix and a TaqMan™ Gene Expression Assay.

Eliminating the RNA isolation step substantially expedites and simplifies gene expression analysis of cultured cells. Cells-to-CT™ Express lysates exhibit sensitivity and specificity similar to that of purified RNA in RT-qPCR. The lysis procedure simultaneously prepares cell lysates for RT-qPCR and removes genomic DNA, in only five 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, for cells grown in 96- or 384-well plates, no sample transfers.

Contents and storage

Component	Cat. no. A57985 (40 rxn)	Cat. no. A57986 (100 rxn)	Cat. no. A57987 (400 rxn)	Cat. no. A57988 (2500 rxn)	Storage
Express ezDNase™	20 µL	50 µL	200 µL	1.25 mL	-20°C
SuperScript™ IV VILO™ Master Mix ^[1]	220 µL	550 µL	2 × 1.1 mL	13.8 mL	
SuperScript™ IV VILO™ No RT Control ^[2]	200 µL	500 µL	1.1 mL	7 mL	
Express Lysis Solution	2 × 1 mL	5 mL	20 mL	125 mL	Room Temperature ^[3]
TaqMan™ Fast Advanced Master Mix	1 mL	5 mL	4 × 5 mL	125 mL	4°C

^[1] Additional SuperScript™ IV VILO™ Master Mix is available for purchase if RT reaction sizes larger than 25 µL are required (Cat. Nos. [11756050](#) or [11756500](#)).

^[2] Additional SuperScript™ IV VILO™ No RT Control is available for purchase if RT reaction sizes larger than 25 µL are required (Cat. Nos. [11756050](#) or [11756500](#)).

^[3] Room Temperature can range from 15 to 25°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.

Catalog numbers that appear as links open the web pages for those products.

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 or 5 Real-Time PCR System	A31668 , A31671
QuantStudio™ 6 and 7 Flex Real-Time PCR System	4485697 , 4485698
QuantStudio™ 12K Flex Real-Time PCR System	4471050
Equipment	
Vortex mixer	MLS
Microcentrifuge	MLS

(continued)

Item	Source
Pipettes	MLS
Consumables	
Nuclease-free pipette tips	MLS
Nuclease-free microcentrifuge tubes	MLS
U-bottom multi-well plates ^[1]	MLS
Real-time PCR tubes or multi-well plates appropriate for instrument	MLS
Reagents	
Nuclease-free water	AM9932
1X PBS, pH 7.4	10010023

^[1] For cells not adhered in 96- or 384-well plates

Workflow

Prepare cells for lysis

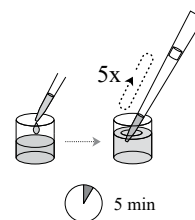
Adherent cells in 96- or 384-well plates: Remove media and wash cells with 1X PBS, then aspirate PBS.

Cells grown in other vessels (adherent or suspension): Remove media and wash cells in 1X PBS, resuspend in 1X PBS at 10^4 to 10^5 cells per 5 μ L. Transfer 5 μ L of the cell suspension to a 96-well plate.



Prepare the Cells-to-CT™ Express lysate

Add 50 μ L of Express Lysis Solution to each well, pipette 5 times or use an orbital shaker to mix. Incubate at room temperature for 5 minutes.



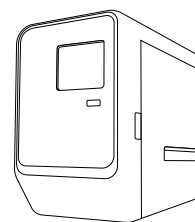
Perform reverse transcription

Combine the SuperScript™ IV VILO™ Master Mix with the lysate, set up the instrument, then run the reaction.



Perform qPCR

Combine the qPCR cocktail with the cDNA, set up the instrument, then run the reaction.



Procedural guidelines

- Using too many cells per lysis reaction can result in incomplete lysis and/or inhibition of RT-qPCR. 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 the Pilot Experiment in “Identify the maximum number of cells” on page 15.
- Use of fresh cultured cells is recommended. 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 Cells-to-CT™ Express lysate” on page 10.
- To avoid bubble formation when mixing the lysate, set the pipette to less than the reaction volume and expel the solution without emptying the pipette tip completely.
- Unless otherwise stated, room temperature is 15–25°C.
- The SuperScript™ IV VILO™ No RT Control contains all the RT reaction components except the reverse transcription enzyme. No RT controls show that the template for the qPCR was cDNA, and not genomic DNA.
- No-template controls (NTC) contain all the qPCR components except the cDNA (substitute water). If the NTC yields a fluorescent signal, the RT or qPCR reagents may be contaminated with DNA, for example, PCR product from previous reactions.

Before you begin

- Upon receiving the TaqMan™ Cells-to-CT™ Express Kit, thaw the TaqMan™ Fast Advanced Master Mix at room temperature. Once thawed, store the TaqMan™ Fast Advanced Master Mix at 4°C until ready to use.

IMPORTANT! TaqMan™ Fast Advanced Master Mix can develop particulates that float in the solution. Such particulates are not a cause for concern and will not affect kit performance.

- Chill 1X PBS to 4°C.
- (Optional) Add Express ezDNase™ (1:100) to Express Lysis Solution, to remove genomic DNA during cell lysis, according to the following table:

Component	1 reaction	96 reactions ^[1]	384 reactions ^[1]
Express Lysis Solution	49.5 µL	5.23 mL	20.91 mL
Express ezDNase™	0.5 µL	52.8 µL	211.2 µL
Total	50 µL	5.28 mL	21.12 mL

^[1] Includes 10% overage

Prepare cells for lysis

Prepare adherent cells grown in 96- or 384-well plates

Use cells that have been cultured until they are fully adhered to the plate to avoid losing cells during the wash.

1. Ensure each well contains 10–10⁵ cells.
2. Aspirate then discard the culture medium from each well.
3. Add a volume of 4°C PBS equal to the original culture media volume to each well.
4. Aspirate then discard the PBS from each well.

Note: Remove as much media and PBS as possible without disturbing the cells.

Prepare 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.

Note: If trypsin is used, be sure to inactivate it before proceeding.

2. Count the cells, then gently centrifuge to collect them at the bottom of the tube.
3. Aspirate and discard the medium, then place the cells on ice.
4. Wash the cells by resuspending them in 500 µL of 4°C PBS per 10⁶ 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 (10–10⁵ cells/reaction).
7. Split the cell suspension into individual lysis reactions (5 µL per reaction) in multi-well plate or individual microcentrifuge tubes, then place on ice.

Prepare Cells-to-CT™ Express lysate

1. Add 50 µL of Express 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 Express ezDNase™ to the Express Lysis Solution, see “Before you begin” on page 8.

Note: Gentle shaking on an orbital shaker provides a more uniform lysis and is the recommended method for plates.

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

STOPPING POINT Lysates can be stored on ice for ≤ 5 hours. Lysates with high cell densities (10⁵) can also be stored at room temperature for ≤ 30 minutes and lysates with lower cell densities (≤ 10⁴) for ≤ 1 hour. For longer term storage, lysates can be stored at -80°C for ≤ 1 year.

Perform reverse transcription

1. In a nuclease-free microcentrifuge tube on ice, prepare enough RT cocktail for the number of 25 µL reactions required, according to the following table:

Component	1 reaction	96 reactions ^[1]	384 reactions ^[1]
5x SuperScript™ IV VILO™ Master Mix ^[2]	5 µL	0.53 mL	2.11 mL
Nuclease-free water	15 µL	1.59 mL	6.34 mL
Total	20 µL	2.12 mL	8.45 mL

^[1] Volumes include 10% overage.

^[2] For the minus-RT control, use SuperScript™ IV VILO™ No RT Control instead of SuperScript™ IV VILO™ Master Mix.

Note: Up to 45% of the RT reaction volume (11.2 µL) can be Cells-to-CT™ Express lysate. We recommend 20% (5 µL, shown here). Adjust the volume of nuclease-free water accordingly.

Note: If 25 µL of cDNA will not provide sufficient material for all the planned qPCR reactions, the RT reaction can be scaled up proportionally. If RT reaction sizes larger than 25 µL are required, SuperScript™ IV VILO™ Master Mix and SuperScript™ IV VILO™ No RT Control are available for purchase in two kit sizes (Cat. Nos. [11756050](#) and [11756500](#)).

2. Mix the RT cocktail gently but thoroughly, then briefly centrifuge and place on ice.

3. Distribute 20 μL of RT cocktail to nuclease-free PCR tubes or wells of a multi-well PCR plate.
4. Add 5 μL of sample lysate to each tube or well of RT cocktail for a final 25- μL reaction volume.

Note: Mix the lysates prior to adding an aliquot into the RT reaction if they were stored, at room temperature, on ice or frozen.

5. Mix reactions gently, then close the tubes or seal the plate. Centrifuge briefly to collect the contents at the bottom of the reaction vessel.

STOPPING POINT Assembled RT reactions can be stored on ice for up to 9 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
Anneal Primers	1	1	25°C	10 minutes
Reverse transcription	2	1	50°C	10 minutes
Inactivate enzyme	3	1	85°C	5 minutes
Hold	4	1	4°C	Indefinite

STOPPING POINT Completed RT reactions can be stored at -80°C for ≤ 1 year.

Perform qPCR

1. In a nuclease-free microcentrifuge tube, prepare the qPCR cocktail for the number of reactions required according to the following table.

Note: For multiple reactions, include 10% overage when making a cocktail.

Component	10 μL reaction	20 μL reaction
2X TaqMan™ Fast Advanced Master Mix	5 μL	10 μL
20X 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 qPCR volume can be cDNA, we recommend 20% (shown here). Adjust the volume of nuclease-free water accordingly.

2. Mix the qPCR cocktail gently but thoroughly, then briefly centrifuge.
3. Distribute the qPCR cocktail into individual PCR tubes or wells of a real-time PCR plate at room temperature.

4. Add cDNA to the qPCR cocktail according to the following table.

Component	10 μ L reaction	20 μ L reaction
qPCR cocktail	8 μ L	16 μ L
cDNA (RT reaction)	2 μ L	4 μ L

5. Close the tubes or seal the plate, then mix gently by vortex mixer.
6. Centrifuge briefly to remove bubbles then collect the contents at the bottom of the tubes/wells.
7. Set up the real-time PCR instrument as indicated in the following table, then load into the instrument and run the reactions.

Note: Specify the fluorescent dye used in the TaqMan™ Gene Expression Assay for the experiment. For more details, refer to the *TaqMan™ Fast Advanced Master Mix User Guide* (Pub. No. MAN0025706).

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

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



Troubleshooting

Observation	Possible cause	Recommended action
No PCR product or unexpected PCR products	The 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 Express 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 $\leq 10^5$ cells can be used successfully in the Cells-to-CT™ Express procedure, but if RT or qPCR fails, try using 5– to 10-fold fewer cells.
		Perform a pilot experiment to determine the optimal number of cells for your cell type. See Appendix B, “Supplemental information”.
		Suboptimal primer design can become apparent at higher cell inputs.
	RNase in the sample was not inactivated: Too much media or PBS was left on the cells, diluting the Express Lysis Solution.	Remove as much culture media as possible prior to PBS wash to remove inhibitors. A second PBS wash can be added to the procedure to remove even more inhibitors. If $> 5 \mu\text{L}$ of PBS remains in the samples when the Express Lysis Solution is added, the Express Lysis Solution is too diluted to inactivate cellular RNases. Remove as much PBS as possible before adding Express Lysis Solution to the cells, or if you split the cells after the PBS wash, resuspend cells in $\leq 5 \mu\text{L}$ PBS for each sample of 10^4 – 10^5 cells.
	Lysates sat too long before going into RT.	Do not allow lysates to sit longer than 30 minutes at room temperature if using high cell densities (10^5) or longer than 1 hour if using lower cell densities ($\leq 10^4$). Either freeze the lysates at -80°C , or start the RT reactions. Alternatively, lysates can be safely stored on ice for up to 5 hours after lysis.

Observation	Possible cause	Recommended action
No PCR product or unexpected PCR products (continued)	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. - 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. - 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-CT™ Express lysate.
	Inhibitors in the RT reaction or PCR.	Check for the presence of RT or PCR inhibitors in the Cells-to-CT™ Express lysate by performing a PCR amplification using a validated assay, housekeeping gene, or by purifying the RNA from the lysate using a standard method. Compare the RT-qPCR results from the purified RNA to that from the lysate.
RT-PCR products in the negative control reactions	PCR products in the no-template PCR control.	Decontaminate pipets and benchtops with DNAZap™ PCR DNA Degradation Solutions (Cat. No. AM9890) or another DNA decontamination product. Careful laboratory practices are essential to avoid contaminating reactions with PCR products. Keep concentrated DNA solutions away from the area where PCR reactions are assembled. Use barrier tips to pipet PCR reagents, and store completed PCR reactions 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 Express ezDNase™ is thoroughly mixed into the Express Lysis Solution for efficient removal of genomic DNA from samples. - Use TaqMan™ Gene Expression Assay 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 Express Lysis Solution that is at room temperature and ensure that lysis reactions occur at room temperature (15–25°C).



Supplemental information

Pilot Experiment

The purpose of this pilot experiment is to identify the maximum number of cells to use in TaqMan™ Cells-to-CT™ Express Kit reactions. Using too many cells can result in inefficient cell lysis and/or RT-qPCR inhibition. The maximum number of cells that can be used with the procedure can vary somewhat according to the cell type.

In this simple experiment, cells are serially diluted and lysed following the standard protocol. The lysates are then subjected to RT-qPCR for an endogenous control gene, such as β -actin, and the C_T values are plotted against the log of the number of cells contained in the lysis reaction. The resulting line will be linear for cell numbers that are compatible with the procedure and will deviate from linearity at concentrations that result in incomplete lysis or RT-qPCR inhibition.

Identify the maximum number of cells

1. Harvest, count, and wash cells. Follow the instructions for harvesting cells, see “Prepare cells grown in other vessels, including adherent and suspension cells” on page 9.
2. Prepare a cell suspension containing 2×10^5 cells/ μ L using cold PBS. Keep the cell suspension on ice.
3. To make 5 serial dilutions of cells in 10-fold increments, prepare 5 tubes containing 45 μ L of cold PBS in ice.
4. Transfer 5 μ L of the 2×10^5 cells/ μ L to the first tube (1:10 dilution) then mix gently but thoroughly.
5. Continue making the serial dilutions by transferring 5 μ L of each solution to the subsequent tube to finish with 5 suspensions containing 2×10^4 , 2000, 200, 20, and 2 cells/ μ L.

IMPORTANT! Use a new pipette tip for each transfer.

6. Transfer 5 μ L of each cell suspension to individual reaction tubes or wells of a multi-well plate. Include 3 biological replicates of each cell concentration. The final cell counts will be 10^5 , 10^4 , 1000, 100, and 10 cells.
7. Add 50 μ L of Express Lysis Solution to each of the samples. See “Before you begin” on page 8.
8. Mix thoroughly by pipetting up and down 5 times, then incubate at room temperature for 5 minutes.

9. To reverse transcribe the RNA and perform qPCR on the samples, follow the protocols for “Perform reverse transcription” on page 10 and “Perform qPCR” on page 11.

Note: Evaluate real-time qPCR of the cell dilution using the TaqMan™ Gene Expression Assay for the target-of-interest to help determine the minimum number of cells required for its detection.

10. To evaluate the results, create a plot of C_T values versus the log of the number of cells in the lysis reaction.

Note: The C_T values should decrease in a linear fashion as the number of cells increase, for cell numbers that are compatible with the procedure. When the number of cells per lysis reaction exceeds the capacity of the system data will not be linear, resulting in lower r^2 values. An r^2 value > 0.98 is generally desirable for RT-qPCR experiments. In future experiments, do not exceed the number of cells per lysis reaction that provided results within the linear range in the pilot experiment.

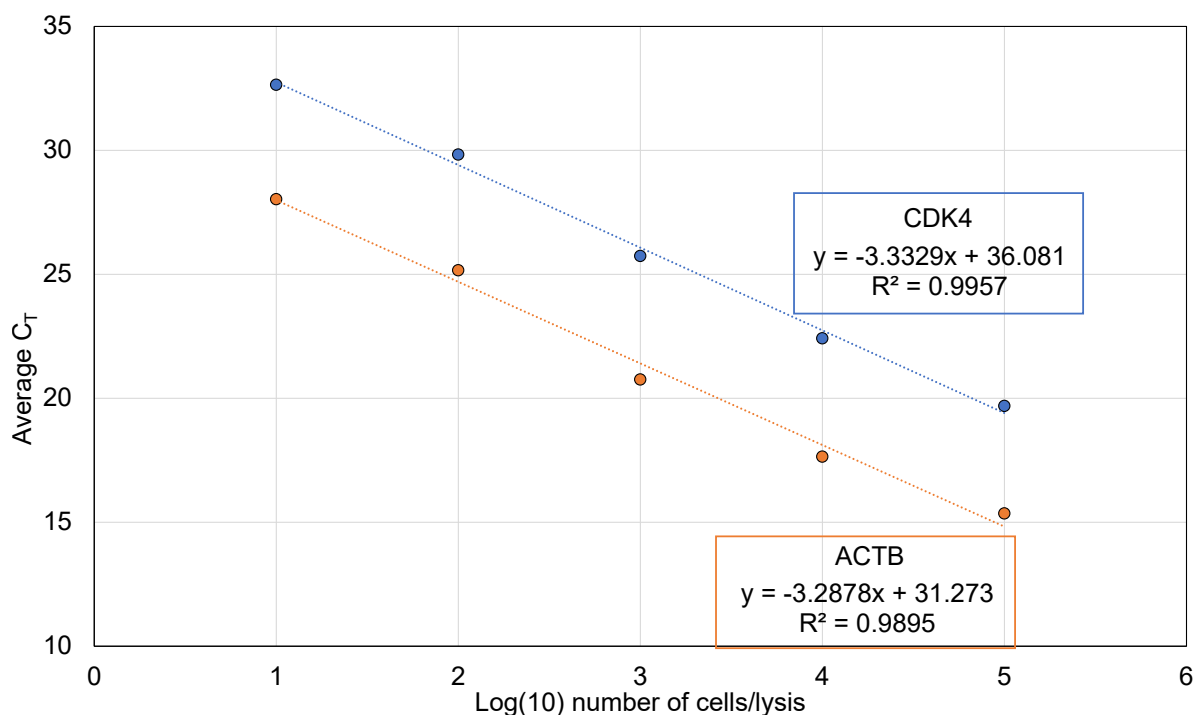


Figure 1 Pilot Experiment Example Data The pilot experiment protocol was used to evaluate the maximum number of human cervical cancer cells that can be used with the TaqMan™ Cells-to-CT™ Express Kit. TaqMan™ Gene Expression Assays were used to assay expression of genes CDK4 (blue) and ACTB (orange). 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 reaction.



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](https://www.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 sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer 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 needed) 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.



WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.



WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.



Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



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)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
[cdc.gov/labs/bmbi](https://www.cdc.gov/labs/bmbi)
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
[who.int/publications/i/item/9789240011311](https://www.who.int/publications/i/item/9789240011311)



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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 its affiliates 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 questions, contact Life Technologies at www.thermofisher.com/support.

