invitrogen

Growth and Maintenance of T-REx™ Cell Lines

USER GUIDE

Catalog Numbers R71007, R71407, R71807, R72207

Publication Number MAN0000106

Revision 5.0

ThermoFisher SCIENTIFIC

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.

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

Revision history: Pub. No. MAN000106

Revision	Date	Description
5.0	22 April 2020	Updated the amount of cells supplied from 3 x 10e6 to 1 x 10 e7. Removed dashes from skus.
4.0	25 February 2020	Updated to current brand standards and added information about storage.
3.0	26 September 2013	Baseline for this revision.

Contents

Product information

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

Product description

The Tetracycline-Regulated Expression (T-REx™) cell lines are intended for generation of stable cell lines for tetracycline-inducible expression of genes of interest with the T-REx™ Expression System. The cell lines stably express the Tet repressor from the pcDNA™6/TR plasmid, which saves significant time and effort when using the T-REx™ system.

Transfect a T-REx™ cell line with one of the vector-based expression constructs containing the gene of interest (Table 1). Induce expression of the gene by adding tetracycline to the cells. Generate stable, inducible cell lines expressing the gene of interest by transfection and dual selection using the vector-specific antibiotic and blasticidin. Blasticidin is used to select for maintenance of the pcDNA™6/TR plasmid.

Table 1 Vectors for tetracycline-inducible gene expression in T-REx™ cell lines

Vector[1]	Vector selection
pcDNA™4/TO	Zeocin™ Selection Reagent
pcDNA™5/TO	Hygromycin
pT-REx-DEST	Geneticin™ Selective Antibiotic

[1] See Appendix B, "Related products".

Parental cell lines

For more information about the parental cell lines, go to **www.atcc.org**.

Table 2 Parental cell lines for T-REx™ cell lines

T-REx™ cell line	Parent cell line	Characteristic	ATCC[1] number
T-REx™-293	293	Adherent	CRL-1573
T-REx™-HeLa	HeLa	Adherent	CCL-2
T-REx™-CHO	CHO-K1	Adherent	CCL-61
T-REx™-Jurkat	Jurkat	Suspension	TIB-152

[1] American Type Culture Collection

Contents and storage

Table 3 T-REx™ cell lines

Cell line	Cat. No.	Amount	Storage
T-REx™-293	R71007	1 vial 1 X 10^7 cells in 1 mL of: 45% complete medium/45% conditioned complete medium/10% DMSO	Store in liquid nitrogen, vapor-phase upon receipt. Shipped on dry ice.
T-REx™-HeLa	R71407		
T-REx™-CHO	R71807		
T-REx™-Jurkat	R72207		

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.
- Prior to starting experiments, ensure you have established cells and have frozen stocks on hand. Upon receipt, grow and freeze multiple vials of cells to ensure that you have an adequate supply of early-passage cells.

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.

Table 4 Materials for general cell culture

Item	Source
Equipment, plastics, and consumables	
Table-top centrifuge	MLS
Sterile flasks, 75-cm^2 and 175-cm^2	MLS
Sterile flasks or plates, other sizes as needed	MLS
Sterile conical tubes, 15-mL	MLS
Sterile pipettes, 5-, 10-, and 25-mL	MLS
Hemacytometer, for counting cells	MLS
Cryovials, for freezing cells	MLS

Item	Source
Reagents	
Phosphate-Buffered Saline (PBS)	See "Prepare Phosphate-Buffered Saline (PBS)" on page 16
0.4% Trypan blue in PBS, for counting cells	MLS
Trypsin-EDTA solution or other trypsin solution	25300054

Table 5 Reagents for T-REx™ cell line media preparation

Item	Source
Dulbecco's Modified Eagle Medium (D-MEM; high glucose)	11965092
Minimum Essential Medium with Earle's Salts (E-MEM)	10370021
Ham's F12	11765054
RPMI 1640 Medium	11875093
Fetal bovine serum	
L-glutamine, 200 mM	25030081
Penicillin-Streptomycin	15070063
Blasticidin	R21001
DMSO	MLS

Table 6 Materials for transfection

Item	Source
Transfection reagents	
Lipofectamine™ 2000 Transfection Reagent	11668019
DMRIE-C	10459014
Antibiotics for vector selection	
Zeocin™ Selection Antibiotic[1]	R25001
Hygromycin-B[2]	10687010
Geneticin® Selective Antibiotic[3]	10131035

[1] ForpcDNA™4/TO constructs.
[2] For pcDNA™5/TO constructs.
[3] For pT-REx-DEST constructs.

Media for cell lines

Cell line	Complete medium	Antibiotic	Freezing medium
T-REx™-293	DMEM (high glucose) 10% FBS[1] 2 mM L-glutamine *(Optional)* 1% Pen-Strep	5 µg/mL blasticidin[2]	45% complete medium 45% conditioned complete medium 10% DMSO
T-REx™-HeLa	EMEM 10% FBS[1] 2 mM L-glutamine *(Optional)* 1% Pen-Strep	5 µg/mL blasticidin[2]	45% complete medium 45% conditioned complete medium 10% DMSO
T-REx™-CHO	Ham's F12 10% FBS[1] 2 mM L-glutamine *(Optional)* 1% Pen-Strep	10 µg/mL blasticidin[2]	45% complete medium 45% conditioned complete medium 10% DMSO
T-REx™-Jurkat	RPMI 1640 10% FBS[1] 2 mM L-glutamine *(Optional)* 1% Pen-Strep	10 µg/mL blasticidin[2]	45% complete medium 45% conditioned complete medium 10% DMSO

[1] Fetal bovine serum.
[2] Required to maintain the pcDNA™6/TR plasmid.

Methods

Culture T-REx cell lines

Guidelines for general T-REx™ cell line handling

- FBS does not need to be heat inactivated for use with these cell lines.
- Many lots of fetal bovine serum (FBS) might contain tetracycline, because FBS is generally isolated from cows that have been fed a diet containing tetracycline. If you culture the cells in medium with FBS that contains tetracycline, you may observe low basal levels of expression from your gene of interest. We have cultured the T-REx™ cell lines in medium with FBS that contains tetracycline, and have observed undetectable to very low basal expression of β-galactosidase from pcDNA™4/TO/*lacZ*.

  If your gene of interest produces a toxic protein, you might wish to culture the T-REx™ cell line in tetracycline-reduced FBS. For more information, consult the supplier of your serum.
- Maintain cell lines in medium containing blasticidin as described in "Media for cell lines" on page 8, to select for the pcDNA™6/TR plasmid.
- If adherent cells (T-REx™-293, T-REx™-HeLa, or T-REx™-CHO) are split at a 1:5 to 1:10 dilution, they will generally reach 80–90% confluence in 3–4 days.
- All solutions and equipment that come in contact with the cells must be sterile. Always use proper sterile technique and work in a laminar flow hood.
- Before starting experiments, be sure to have cells established and also have some frozen stocks on hand. We recommend using early-passage cells for your experiments. Upon receipt of the cells, grow and freeze multiple vials of the particular cell line to ensure that you have an adequate supply of early-passage cells.
- For general maintenance of cells, pass all cell lines when they are 80–90% confluent (for adherent cells) or when they reach a density of 5×10^6 to 1×10^7 cells/mL (for suspension cells).
- Use trypan blue exclusion to determine cell viability. Log phase cultures should be >90% viable.

Before you begin

Prepare the appropriate complete medium; see "Media for cell lines" on page 8.

Thaw adherent cells

Follow this protocol to thaw adherent cells to initiate cell culture. All cell lines are supplied in vials containing 1×10^7 cells/mL.

1. Remove the vial of cells from the liquid nitrogen and thaw quickly at 37°C.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol, and transfer the cells to a sterile, conical tube containing 5 mL of complete medium without antibiotic.
3. Centrifuge for 3 minutes at 750 × *g* at room temperature.
4. Aspirate off the medium and resuspend the cells in 12 mL of fresh, complete medium without antibiotic.
5. Transfer the cells to a T-75 flask and incubate cells overnight at 37°C.
6. The next day, aspirate off the medium and replace with fresh, complete medium containing the appropriate antibiotic (see “Media for cell lines” on page 8.
7. Incubate the cells and check them daily until the cells are 80–90% confluent (2–7 days).
8. Proceed to“Passage adherent cells” on page 11.

Thaw suspension cells

Follow this protocol to thaw suspension cells to initiate cell culture. All cell lines are supplied in vials containing 1×10^7 cells/mL.

1. Remove the vial of cells from the liquid nitrogen and thaw quickly at 37°C.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol, and transfer the cells to a sterile, conical tube containing 5 mL of complete medium without antibiotic.
3. Centrifuge for 3 minutes at 750 × *g* at room temperature.
4. Aspirate off the medium and resuspend the cells in 12 mL of fresh, complete medium without antibiotic.
5. Transfer the cells to a T-75 flask and incubate cells overnight at 37°C.
6. The next day, add antibiotic to the cells (see “Media for cell lines” on page 8).
7. Incubate the cells and count them daily until the cells reach a density ranging from 5×10^6 cells/mL to 1×10^7 cells/mL (2–7 days).

   Note: You may add fresh, complete medium containing antibiotic to the cells every few days.
8. Proceed to “Passage suspension cells” on page 11.

Passage adherent cells

1. When cells are ~80–90% confluent, remove all medium from the flask.
2. Wash cells once with 10 mL PBS to remove excess medium and serum. Serum contains inhibitors of trypsin.
3. Add 5 mL of trypsin-EDTA solution to the monolayer and incubate 1–5 minutes at room temperature until cells detach. Check the cells under a microscope and confirm that most of the cells have detached. If cells are still attached, incubate a little longer until most of the cells have detached.
4. Add 5 mL of complete medium to stop trypsinization.
5. Briefly pipet the solution up and down to break up clumps of cells.
6. To maintain cells in 75-cm^2 flasks, transfer 1 mL of the 10 mL cell suspension from Step 5 to a new 75-cm^2 flask and add 15 mL fresh, complete medium containing the appropriate concentration of antibiotic (see “Media for cell lines” on page 8).

   Note: If you want the cells to reach confluency sooner, split the cells at a lower dilution (for example. 1:4).
7. To expand cells, add 28 mL of fresh, complete medium to each of three 175-cm^2 flasks, then transfer 2 mL of the cell suspension to each flask to obtain a total volume of 30 mL.
8. Incubate flasks in a humidified, 37°C, 5% CO_2 incubator.

Repeat as necessary to maintain or expand cells.

Passage suspension cells

1. When cells reach the desired density, transfer cells to a sterile, conical centrifuge tube.
2. Centrifuge for 5 minutes at 750 × g at room temperature.
3. Aspirate off the medium and resuspend the cells in 10 mL of fresh, complete medium containing antibiotic.
4. To maintain cells in 75-cm2 flasks, transfer 1 mL of the 10 mL cell suspension from Step 3 to a new 75-cm2 flask and add 15 mL of fresh, complete medium containing the appropriate concentration of antibiotic (see “Media for cell lines” on page 8).

   Note: If you want the cells to reach confluency sooner, split the cells at a lower dilution (for example. 1:4).
5. To expand cells, add 28 mL of fresh, complete medium to each of three 175-cm2 flasks, then transfer 2 mL of the cell suspension to each flask to obtain a total volume of 30 mL.
6. Incubate flasks in a humidified, 37°C, CO2 incubator.

Repeat as necessary to maintain or expand cells.

Freeze T-REx cell lines

Guidelines for freezing T-REx™ cell lines

- Freeze cells at a density of at least 1 × 10^7 cells/mL.
- Use the freezing medium as described in “Media for cell lines” on page 8.

Before you begin

Obtain conditioned complete medium

To prepare freezing medium, you must first obtain conditioned complete medium. Conditioned medium is the medium in which cells have been growing.

Obtain conditioned medium in one of the following ways.

- Harvest the medium from the cells one day before you plan to freeze them, by performing the steps in the following table.
- Harvest the medium from the cells just prior to freezing. Follow the procedure in the table except do not refeed or replate the cells.

Cell type	Procedure
Adherent	**1.** Remove and reserve the medium from the cells. **2.** Refeed the cells with fresh complete medium containing antibiotic. **3.** Store the conditioned medium in a 50 mL sterile, conical centrifuge tube at 4°C until use
Suspension	**1.** Transfer the cells to a sterile, conical tube and centrifuge for 5 minutes at 750 × *g* at room temperature. **2.** Remove and reserve the medium from the cells. **3.** . Add the appropriate amount of fresh complete medium containing antibiotic to the cells to obtain a density of 2–3 × 10^6 cells/mL. Replate the cells. **4.** Store the conditioned medium in a 50 mL sterile, conical centrifuge tube at 4°C until use.

Prepare freezing medium

Prepare freezing medium immediately before use.

1. In a sterile, conical centrifuge tube, mix together the following reagents for every 1 mL of freezing medium needed.

   Table 7

Component	Volume per 1 mL of freezing medium
Fresh complete medium	0.45 mL
Conditioned complete medium	0.45 mL
DMSO	0.10 mL

2. Place the tube on ice until use.

Discard any remaining freezing medium after use.

Freeze cells

Before starting, label cryovials and prepare freezing medium (see “Prepare freezing medium” on page 13). Keep the freezing medium on ice.

1. To collect cells, perform the following: • •
   - For adherent cells, follow Steps 1–5 of “Passage adherent cells” on page 11. If you are making freezing medium, reserve the conditioned medium.
   - For suspension cells, transfer cells to a sterile, conical centrifuge tube.

     Note: If you are making freezing medium, you will need to centrifuge the cells, collect the medium, and resuspend cells in fresh, complete medium.
2. Count the cells.
3. Pellet cells at 250 × *g* for 5 minutes in a table top centrifuge at 4°C, then carefully aspirate off the medium.
4. Resuspend the cells at a density of at least 1×10^7 cells/mL in chilled freezing medium (see “Prepare freezing medium” on page 13).
5. Place vials in a styrofoam microcentrifuge rack and aliquot 1 mL of the cell suspension into each vial. Once vials have been capped, place a second styrofoam rack on top of the vials to provide additional insulation. Transfer vials to –20°C for 2 hours.
6. Transfer vials to a –70°C or –80°C freezer and hold overnight.
7. Transfer vials to liquid nitrogen, vapor-phase, for long-term storage.

Guidelines for transfection

General guidelines for transfection

T-REx™-293 cells, T-REx™-HeLa cells, T-REx™-CHO cells, and T-REx™-Jurkat cells are generally amenable to transfection using standard methods including calcium phosphate precipitation, lipid-mediated transfection, and electroporation. We typically use lipid-mediated transfection to introduce the T-REx™ expression construct containing your gene of interest into T-REx™ cell lines. The following table lists the recommended transfection reagent for each T-REx™ cell line. Other transfection reagents, such as Calcium Phosphate Transfection Kit (Cat. No. K278001) may be suitable.

To transfect...	Use...
T-REx™-293 cells T-REx™-HeLa cells T-REx™-CHO cells	Lipofectamine™ 2000 Transfection Reagent (Cat. No. 11668019)
T-REx™-Jurkat	DMRIE-C (Cat. No. 10459014)

Guidelines for transient transfection

Any of the T-REx™ cell lines may be transfected with the pcDNA™4/TO, pcDNA™5/TO, or pT-REx-DEST-based construct containing your gene of interest and analyzed by transient expression. General guidelines are provided below to transfect your T-REx™ expression construct into any T-REx™ cell line and to induce expression of your protein of interest with tetracycline. For more information about the T-REx™ expression vectors and induction with tetracycline, refer to the T-REx™ System manual.

- To transfect adherent cells, use cells that are approximately 60% confluent.
- To transfect suspension cells, use 1–2 × 10^6 cells (in a 6-well plate). The cell number may vary depending on the size of your tissue culture plate.
- Transfect your T-REx™ expression construct into the T-REx™ cell line using the appropriate method.
- After transfection, allow the cells to recover for 24 hours before induction.
- To induce expression of the gene of interest, we recommend adding tetracycline to a final concentration of 1 µg/mL (5 µL of a 1 mg/mL stock per 5 mL of medium) and incubating the cells for 24 hours at 37°C. Please refer to the T-REx™ System manual for instructions to prepare tetracycline.
- Harvest the cells and assay for expression of your gene.

Guidelines for generation of dual stable cell lines

Stable T-REx™ cell lines that express your gene of interest and the Tet repressor can be generated by transfection of your pcDNA™4/TO, pcDNA™/TO, or pT-REx-DEST-based construct and dual selection with either Zeocin™ (Cat. No. R25001; for pcDNA™4/TO-based constructs), hygromycin (Cat. No. 10687010; for pcDNA™5/TO-based constructs), or Geneticin™ (Cat. No. 10131035; for pT-REx-DEST-based constructs) and blasticidin (Cat. No. R210-01).

IMPORTANT! Before transfection, we suggest that you test the sensitivity of the T-REx™ cell line to Zeocin™, hygromycin, or Geneticin™ antibiotics to determine the appropriate concentration of antibiotic to use for selection.

The following table lists the suggested range of Zeocin™ or hygromycin antibiotic concentrations to use for selection of pcDNA™4/TO or pcDNA™5-based constructs, respectively.

Cell line (after transfection with expression vector)	Estimated Zeocin™ antibiotic concentration (µg/mL)	Estimated hygromycin concentration (µg/mL)
T-REx™-293	200–400	100–300
T-REx™-HeLa	100–200	100–300
T-REx™-CHO	200–400	400–600
T-REx™-Jurkat	200–400	200–400

Recipes

Prepare Phosphate-Buffered Saline (PBS)

For washing cells only

- 137 mM NaCl
- 2.7 mM KCl
- 10 mM Na_2HPO_4
- 1.8 mM KH_2PO_4

1. Dissolve the following in 800 mL deionized water.
   - 8 g NaCl
   - 0.2 KCl
   - 1.44 g Na_2HPO_4
   - 0.25 g KH_2PO_4
2. Adjust pH to 7.4 with concentrated HCl.
3. Bring the volume to 1 L, then autoclave for 20 minutes on liquid cycle.

Store at 4℃ or room temperature.

B

Related products

Additional reagents

The products listed below may be used with the T-REx™ cell lines. Zeocin™, hygromycin, and Geneticin™ antibiotics are available for selection ofpcDNA™4/TO, pcDNA™5/TO, and pT-REx™-DEST-based constructs, respectively, after transfection into T-REx™ cell lines.

Item	Amount	Cat. No.
Blasticin	50 mg	R21001
Zeocin™ Selection Antibiotic	8 x 1.25 mL	R25001
	50 mL	R25005
Hygromycin-B	20 mL	10687010
Geneticin™ Selective Antibiotic	20 mL	10131035
Lipofectamine™ 2000 Transfection Reagent	0.75 mL	11668027
	1.5 mL	11668019
DMRIE-C	1 mL	10459014

Cell culture reagents

Item	Amount	Cat. No.
Dulbecco's Modifified Eagle Medium (D-MEM)	500 mL	11965092
Minimum Essential Medium with Earle’s Salts (E-MEM)	500 mL	10370021
Ham’s F-12	500 mL	11765054
RPMI 1640 Medium	500 mL	11875093
200 mM L-Glutamine Selective Antibiotic	100 mL	25030081
Penicillin-Streptomycin	100 mL	15070063
Trypsin-EDTA	100 mL	25300054

T-REx™ products

The following T-REx™ expression plasmids may be used to inducibly express your gene of interest in the T-REx™ cell lines.

Item	Amount	Cat. No.
pcDNA™4/TO	20 µg	V102020
pcDNA™4/TO/*myc*-His A, B, & C	20 µg each	V103020
pcDNA™5/TO	20 µg	V103320
Gateway™ pTRex-DEST30 Vector	6 µg	12301016
Gateway™ pTRex-DEST31 Vector	6 µg	12302014

Flp-In™ T-REx™ products

The T-REx™ cell lines may be used to generate Flp-In™ T-REx™ host cell lines to inducibly express a gene of interest from a specific genomic location. Flp-In™ T-REx™ host cell lines can be generated by stably introducing the pFRT/*lac*Zeo or pFRT/*lac*Zeo2 plasmid into a T-REx™ cell line.

Item	Amount	Cat. No.
pFRT/*lac*Zeo	20 µg	V601520
pFRT/*lac*Zeo2	20 µg	V602220
pcDNA™5/FRT/TO	20 µg	V652020
pcDNA™5/FRT/TO TOPO TA Expression Kit	20 reactions	K651020
pOG44	20 µg	V600520

C

References

References

Chen, C., and Okayama, H. (1987). High-Efficiency Transformation of Mammalian Cells by Plasmid DNA. Molec. Cell. Biol. *7*, 2745-2752.

Chu, G., Hayakawa, H., and Berg, P. (1987). Electroporation for the Efficient Transfection of Mammalian Cells with DNA. Nucleic Acids Res. *15*, 1311-1326.

Felgner, P. L., Holm, M., and Chan, H. (1989). Cationic Liposome Mediated Transfection. Proc. West. Pharmacol. Soc. *32*, 115-121.

Felgner, P. L. a., and Ringold, G. M. (1989). Cationic Liposome-Mediated Transfection. Nature *337*, 387-388.

Graham, F. L., Smiley, J., Russell, W. C., and Nairn, R. (1977). Characteristics of a Human Cell Line Transformed by DNA from Human Adenovirus Type 5. J. Gen. Virol. *36*, 59-74.

Kao, F. T., and Puck, T. T. (1968). Genetics of Somatic Mammalian Cells, VII. Induction and Isolation of Nutritional Mutants in Chinese Hamster Cells. Proc. Natl. Acad. Sci. USA *60*, 1275-1281.

Shigekawa, K., and Dower, W. J. (1988). Electroporation of Eukaryotes and Prokaryotes: A General Approach to the Introduction of Macromolecules into Cells. BioTechniques *6*, 742-751.

Weiss, A., Wiskocil, R. L., and Stobo, J. D. (1984). The Role of T3 Surface Molecules in the Activation of Human T Cells: A Two-stimulus Requirement for IL2 Production Reflects Events Occurring at a Pre-translational Level. J. Immunol. *133*, 123-128.

Wigler, M., Silverstein, S., Lee, L.-S., Pellicer, A., Cheng, Y.-C., and Axel, R. (1977). Transfer of Purified Herpes Virus Thymidine Kinase Gene to Cultured Mouse Cells. Cell *11*, 223-232.

D

Safety

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

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

Chemical safety

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

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with 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)*, 5th Edition, HHS Publication No. (CDC) 21-1112, Revised December 2009; found at:
  https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2009-P.pdf
- World Health Organization, *Laboratory Biosafety Manual*, 3rd Edition, WHO/CDS/CSR/LYO/2004.11; found at:
  www.who.int/csr/resources/publications/biosafety/Biosafety7.pdf

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

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

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

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale 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**.