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Growth and maintenance of the 293FT cell line

USER GUIDE

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ThermoFisher SCIENTIFIC

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

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Revision	Date	Description
B.0	14 February 2020	Updated contents to state liquid nitrogen vapor phase. Added the Important guidelines for thawing and storing cells topic.
A.0	21 April 2015	Remove RUO statement, add LULL 561, update trademark language and branding

Contents

Growth and maintenance of the 293FT cell line

Contents and storage

293FT cell line

The 293FT Cell Line is used for the production of lentiviral stocks. The 293FT Cell Line is supplied as one vial containing 1×10^7 frozen cells in 1 mL of Freezing Medium. **Upon receipt, store in liquid nitrogen vapor-phase until use.**

CAUTION! Handle as potentially biohazardous material under at least Biosafety Level 2 containment.

WARNING! GENERAL CHEMICAL HANDLING. For every chemical, 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 **www.lifetechnologies.com/support**.

This product contains Dimethyl Sulfoxide (DMSO); components of the product may be absorbed into the body through the skin.

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.

Product information

Product description

293FT cell line

The 293FT Cell Line is a very suitable host for lentiviral production. The 293FT Cell Line is derived from the 293F Cell Line (see **“Parental cell lines” on page 6**) and stably expresses the SV40 large T antigen from the pCMVSPORT6TAg.neo plasmid. Expression of the SV40 large T antigen is controlled by the human cytomegalo-virus (CMV) promoter and is high-level and constitutive. For more information about pCMVSPORT6TAg.neo, see **Appendix A, “Appendix”**.

Use of the cell line

Studies have demonstrated maximal virus production in human 293 cells expressing SV40 large T antigen (Naldini et al., 1996), making the 293FT Cell Line a particularly suitable host for generating lentiviral constructs using the ViraPower™ Lentiviral Expression System available from Thermo Fisher Scientific (Cat. no. K4950-00 and K4960-00).

Parental cell lines

The 293 Cell Line is a permanent line established from primary embryonal human kidney transformed with sheared human adenovirus type 5 DNA (Graham et al., 1977; Harrison et al., 1977). The E1A adenovirus gene is expressed in these cells and participates in transactivation of some viral promoters, allowing these cells to produce very high levels of protein.

The 293-F Cell Line available from Thermo Fisher Scientific (Cat. no. 11625) is a fast-growing variant of the 293 cell line, and was originally obtained from Robert Horlick at Pharmacopeia.

Antibiotic resistance

293FT cells stably express the neomycin resistance gene from pCMVSPORT6TAg.neo and should be maintained in medium containing Geneticin™ Selective Antibiotic at the concentration listed, “Media for 293FT cells” on page 8. Expression of the neomycin resistance gene in 293FT cells is controlled by the SV40 enhancer/promoter.

Methods

Important procedural guidelines

General cell handling

Follow the general guidelines below to grow and maintain 293FT cells.

- **Make sure that all solutions and equipment that come in contact with the cells are 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.
- For general maintenance of cells, pass 293FT cells when they are >80% confluent. Avoid overgrowing cells before passaging.
- Maintain 293FT cells in complete medium containing 500 µg/mL Geneticin™ Selective Antibiotic.
- Use trypan blue exclusion to determine cell viability. Log phase cultures should be >90% viable.
- When thawing or subculturing cells, transfer cells into medium warmed to room temperature.
- Cells should be at the appropriate confluence and at greater than 90% viability prior to transfection (see “Transfect cells” on page 12).

Before starting

Be sure to have the following solutions and supplies available:

- 15-mL sterile, conical tubes
- Appropriate sized tissue culture flasks and pipettes
- Complete medium (see “Media for 293FT cells” on page 8)
- 50 mg/mL Geneticin™ Selective Antibiotic
- Phosphate-Buffered Saline (PBS; Thermo Fisher Scientific, Cat. no. 10010-023)
- Reagents for counting cells
- Trypsin/versene (EDTA) solution or other trypsin solution
- Freezing Medium (see “Media for 293FT cells” on page 8 and **Error! Bookmark not defined.**)
- Table-top centrifuge
- Cryovials (if needed)

Media for 293FT cells

The following table lists the recommended complete medium, freezing medium, and antibiotic concentration required to maintain and culture the 293FT Cell Line.

Note: Fetal bovine serum should not be heat-inactivated for use with the 293FT Cell Line.

Complete medium	Antibiotic	Freezing medium
D-MEM™ (high glucose) 10% fetal bovine serum (FBS) 0.1 mM MEM™ Non-Essential™ Amino Acids (NEAA) 6 mM L-glutamine 1 mM MEM™ Sodium Pyruvate 1% Pen-Strep (optional)	500 µg/mL Geneticin™ Selective Antibiotic	90% complete medium 10% DMSO

Note

D-MEM™ already contains 4 mM L-glutamine, which is enough to support cell growth of the 293FT Cell Line. However, since L-glutamine slowly decays over time, the complete medium needs to be supplemented with 2 mM L-glutamine. This will ensure that the concentration of L-glutamine in complete medium will not get too low over time due to its slow degradation.

Note: 293FT cells grow well in 6 mM L-glutamine, but higher concentrations of L-glutamine may reduce growth.

Prepare medium

Prepare the complete D-MEM™ medium containing 10% FBS supplemented with 0.1 mM MEM™ Non-Essential™ Amino Acids, 1 mM sodium pyruvate and 2 mM L-glutamine as described in this section.

Perform all steps in a tissue culture hood under sterile conditions.

1. Remove 100 mL D-MEM™ from 1 L D-MEM™ bottle and replace with 100 mL FBS.
2. To the bottle of medium, add the following:

200 mM L-Glutamine (100X)	10 mL
10 mM MEM™ Non-Essential™ Amino Acids (100X)	10 mL
100 mM MEM™ Sodium Pyruvate (100X)	10 mL
Optional: Penicillin-Streptomycin (100X)	10 mL

3. Filter sterilize the medium using 0.45 µm filtration device.

4. Store the complete medium at 4℃ until use. The medium is stable for 6 months at 4℃ (avoid introducing any contamination into the medium).

5. To an aliquot of the complete medium, add Geneticin™ Selective Antibiotic to prepare complete medium with 500 µg/mL Geneticin™ Selective Antibiotic.

Thaw cells

Introduction

The 293FT Cell Line is supplied in a vial containing 3×10^6 cells in 1 mL of Freezing Medium. Store frozen 293FT cells in liquid nitrogen vapor-phase until ready to use.

Thaw procedure

Use the following procedure to thaw 293FT cells to initiate cell culture. Thaw cells in prewarmed, complete medium **without** Geneticin™ Selective Antibiotic.

1. Remove the vial of frozen cells from liquid nitrogen and thaw quickly in a 37℃ water bath.

2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol, and transfer the cells to a sterile 15-mL tube containing PBS. Briefly centrifuge the cells at 150–200 × *g* and resuspend the cells in 2 mL complete medium without Geneticin™ Selective Antibiotic.

3. Transfer™ the cells to T-75 cm^2 flask containing 10 mL of complete medium without Geneticin™ Selective Antibiotic.

4. Incubate the flask overnight at 37℃ for allowing the cells to attach to the bottom of the flask.

5. The next day, aspirate off the medium and replace with fresh, complete medium containing 500 µg/mL Geneticin™ Selective Antibiotic.

6. Incubate the cells and check them daily until the cells are 80–90% confluent.

7. Proceed to **Subculturing Cells**.

Note: We recommend subculturing cells for a minimum of 3 passages after thawing before use in other applications.

Subculture cells

Introduction

Follow the recommendations and procedures in this section to subculture 293FT cells. Maintain cells as adherent monolayer cultures in complete medium containing 500 µg/mL Geneticin™ Selective Antibiotic.

Subculture conditions

Use the following recommended conditions to subculture 293FT cells. For a procedure to subculture cells, see the following table.

Parameter	Recommended condition
Cell density	>5 × 10^5 viable cells/mL (>80% confluent)
Culture vessel	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15–20 mL for T-75 cm^2 flasks and 40–50 mL for T-162 cm^2 flasks
Seeding density	2–5 × 10^4 viable cells/cm^2
Incubation conditions	37°C incubator with a humidified atmosphere of 5–10% CO_2 in air; loosen caps to allow for oxygenation/aeration

Determine cell density and viability

Follow the provided procedure to determine viable and total cell counts using the trypan blue exclusion method.

1. Transfer™ a small aliquot of the cell suspension to a microcentrifuge tube and dilute the cells such that the total number of cells counted will not be <100 or > 1000.
2. To 1 mL of the diluted cell suspension, add 100 µL Trypan Blue Stain (0.4%) solution. Gently aspirate to mix.
3. Record the dilution factor. The dilution factor equals the total volume (amount of cell suspension and amount of trypan blue) divided by the amount of cell suspension.
4. Incubate the cells with the trypan blue solution for 1–2 minutes.
5. Count all cells (including the blue cells) using a Countess™ Automated Cell Counter (Thermo Fisher Scientific, Cat. no. C1027) or manually using a hemocytometer chamber.
6. To calculate the total cells per mL in suspension, multiply the total count by the dilution factor.
7. To determine the viability, count only the blue cells. Calculate the % viability:
   [1.00 – (Number of blue cells ÷ Number of total cells)] × 100
8. Cell viability should be ≥95% for healthy log-phase cultures.

Subculture procedure

Use this procedure to subculture 293FT cells grown in a T-75 cm^2 flask. If you are using other-sized flasks, scale the reagent volumes accordingly.

1. Remove all medium from the flask and wash the cells once with 10 mL PBS to remove excess medium and serum. Serum contains inhibitors of trypsin.
2. Add 2 mL of trypsin/versene (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.
3. Add 8 mL complete medium containing Geneticin™ Selective Antibiotic and transfer the cell suspension to a 15-mL sterile, conical tube.
4. Determine viable and total cell counts (see “Determine cell density and viability” on page 10).
5. Seed cells at the recommended density (see table in “Subculture conditions” on page 10), diluting in pre-warmed complete medium containing 500 µg/mL Geneticin™ Selective Antibiotic. Incubate flasks as recommended (see table).
6. Maintain cells as adherent monolayer cultures in complete medium containing 500 µg/mL Geneticin™ Selective Antibiotic.
7. For the transfection protocol, you will need 6×10^6 293FT cells for each sample (“Transfect cells” on page 12).

Cryopreservation

Introduction

Once you have established the cells, we recommend freezing some cells for future use as described in the following section.

- Freeze cells at a density of **at least** 3×10^6 viable cells/mL.
- Use a freezing medium composed of 90% complete medium and 10% DMSO. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and store at 4℃ until use. Discard any remaining freezing medium after use.

Freeze cells

Before starting, label cryovials and prepare freezing medium ("Media for 293FT cells" on page 8). Keep the freezing medium on ice.

1. Culture the desired quantity of 293FT cells to 70–90% confluence.
2. Remove the cells from the tissue culture flask(s) following Steps 1 on page 12–3 on page 12, "Subculture procedure" on page 11.
3. Determine viable and total cell counts (see procedure in "Determine cell density and viability" on page 10) and calculate the volume of freezing medium required to yield a final cell density of $\geq 3 \times 10^6$ cells/mL.
4. Prepare the required volume of freezing medium determined in step 2 on page 12.
5. Centrifuge the cells suspension (from Step 2 on page 12) at 250 × *g* for 5 minutes in a table top centrifuge at room temperature. Carefully aspirate off the medium and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
6. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications.
7. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
8. Transfer™ vials to liquid nitrogen storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing cryovials in liquid nitrogen vapor-phase by following the procedure outlined in **"Thaw procedure" on page 9**.

Transfect cells

Transfection methods

The 293FT Cell Line is generally amenable to transfection using standard methods including calcium phosphate precipitation (Chen & Okayama, 1987; Wigler et al., 1977), lipid-mediated transfection (Felgner et al., 1989; Felgner & Ringold, 1989), and electroporation (Chu et al., 1987; Shigekawa & Dower, 1988). We typically use cationic lipid-based transfection reagents to transfect 293FT cells. Lipofectamine™ 2000 Transfection Reagent is recommended, but other transfection reagents are suitable. Lipofectamine™ 2000 Transfection Reagent is available from Thermo Fisher Scientific (see "Accessory products" on page 15 for ordering information).

Transient transfection

The 293FT Cell Line may be transiently transfected with any plasmid. General guidelines are provided below.

- Make sure that cells are healthy at the time of plating. Overgrowth of cells prior to passaging can compromise their transfection efficiency.
- On the day before transfection, plate cells such that they will be at the appropriate confluence at the time of transfection (see manufacturer's recommendations for the transfection reagent you are using). **Example:** If you are using Lipofectamine™ 2000 Reagent as a transfection reagent, plate cells such that they will be 90–95% confluent at the time of transfection.
- Transfect your plasmid construct into the 293FT Cell Line using the method of choice (see "Transfection methods" on page 12).
- After transfection, add fresh growth medium containing 500 µg/mL Geneticin™ Selective Antibiotic and allow the cells to recover for 24–48 hours before proceeding to assay for expression of your gene of interest.

Stable cell line generation

293FT cells can be used as hosts to generate a stable cell line expressing your gene of interest from most plasmids (see the following **"Note" on page 13**). Remember that the introduced plasmid must contain a selection marker other than neomycin resistance. Stable cell lines can then be generated by transfection and dual selection with Geneticin™ Selective Antibiotic and the appropriate selection agent.

Note

Since 293FT cells stably express the SV40 large T antigen, we **do not** recommend generating stable cell lines with plasmids that contain the SV40 origin of replication.

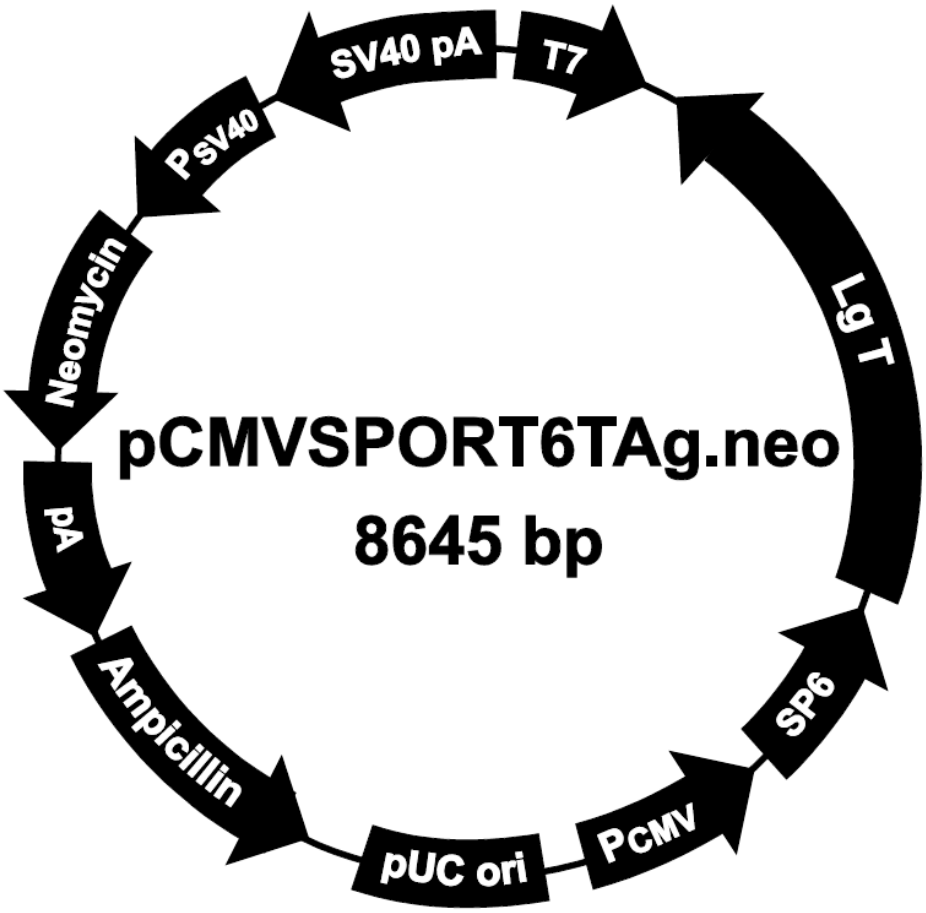
Appendix

Map of pCMVSPORT6Tag.neo

Description

The pCMVSPORT6Tag.neo plasmid is derived from pCMVSPORT6, which has been modified to include the following features:

- The neomycin resistance gene for stable selection in mammalian cells (Southern & Berg, 1982). Expression of the neomycin resistance gene is controlled by the SV40 early enhancer/promoter from which the SV40 origin of replication has been removed.
- The gene encoding the SV40 large T antigen to facilitate optimal virus production (e.g., Thermo Fisher Scientific's ViraPower™ Lentiviral Expression System) and to permit episomal replication of plasmids containing the SV40 early promoter and origin. Expression of the SV40 large T antigen is controlled by the human cytomegalovirus (CMV) promoter.



Accessory products

Accessory products

The products listed in the following table may be used with the 293FT Cell Line. For more information, refer to our website (**www.lifetechnologies.com**) or call Technical Support (see).

Note: Some reagents are available in other sizes.

Item	Amount	Catalog no.
Lipofectamine™ 2000 Transfection Reagent	0.75 mL	11668-027
	1.5 mL	11668-019
Dulbecco's Modified Eagle Medium (D-MEM™)	500 mL	11965-092
	1000 mL	11965-084
Fetal Bovine Serum	100 mL	16000-036

Item	Amount	Catalog no.
Fetal Bovine Serum	500 mL	16000-044
10 mM MEM™ Non-Essential™ Amino Acids Solution	100 mL	11140-050
200 mM L-Glutamine	100 mL	25030-081
MEM™ Sodium Pyruvate Solution (100X)	100 mL	11360-070
Penicillin-Streptomycin	100 mL	15070-063
Trypsin-EDTA	100 mL	25300-054
Geneticin™ Selective Antibiotic	1 g	11811-023
	5 g	11811-031
	20 mL (50 mg/mL)	10131-035
	100 mL (50 mg/mL)	10131-027
Opti-MEM™ I Reduced Serum Medium	100 mL	31985-062
	500 mL	31985-070
Phosphate-Buffered Saline (PBS), pH 7.4	500 mL	10010-023
	1 L	10010-031
Trypan Blue Stain	100 mL	15250-061
Countess™ Automated Cell Counter	1 each	C10227

References

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

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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

Southern, P. J., and Berg, P. (1982) Transformation of Mammalian Cells to Antibiotic Resistance with a Bacterial Gene Under Control of the SV40 Early Region Promoter. J. Molec. Appl. Gen. *1*, 327-339

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

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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

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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**.