USER GUIDE

invitrogen™
by life technologies™

Growth and Maintenance of T-REx™ Cell Lines

Catalog Numbers R710-07, R714-07, R718-07, R722-07

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Contents

Product information

Contents and Storage

Shipping and storage

All cell lines are shipped on dry ice. Store in liquid nitrogen upon receipt.

Contents

This manual is supplied with the following cell lines:

Cell Line	Cat. no.
T-REx™-293	R710-07
T-REx™-HeLa	R714-07
T-REx™-CHO	R718-07
T-REx™-Jurkat	R722-07

All cell lines are supplied as one vial containing 3×10^6 cells in 1 mL of 45% complete medium, 45% conditioned complete medium, and 10% DMSO.

Description of the system

T-REx™ cell lines

The Tetracycline-Regulated Expression (T-REx™) cell lines stably express the tetracycline (Tet) repressor and are designed for use with the T-REx™ System (Catalog nos. K1020-01, K1020-02, K1030-01, and K1030-02). See the following table for information about the generation of the T-REx™ cell lines. For more information about the T-REx™ System and its components, refer to the T-REx™ System manual, visit **www.lifetechnologies.com**, or contact Technical Support (see page 17).

For expression in the T-REx™ cell lines, transfect the cells with a pcDNA™4/TO, pcDNA™5/TO, or pT-REx-DEST-based expression construct containing your gene of interest. Induce expression of your gene of interest by adding tetracycline to the cells. Generate stable, inducible cell lines expressing your gene of interest from a pcDNA™4/TO, pcDNA™5/TO, or pT-REx-DEST-based expression vector by transfection and dual selection using Zeocin™, hygromycin, or Geneticin® antibiotics, respectively, and blasticidin.

Parental cell lines

The table below provides a brief description of the source of the parental cell line used to generate each T-REx™ cell line. The parental cell lines were obtained from the American Type Culture Collection (ATCC). The ATCC number for each cell line is included. For further information about the parental cell lines, refer to the ATCC website (**www.atcc.org**).

Cell line	Characteristic	Source	ATCC number
293	Adherent	Human embryonic kidney (Graham et al., 1977)	CRL-1573
HeLa	Adherent	Human cervical adenocarcinoma	CCL-2
CHO-K1	Adherent	Chinese hamster ovary (Kao and Puck, 1968)	CCL-61
Jurkat	Suspension	Human T-cell leukemia (Weiss et al., 1984)	TIB-152

Generate the T-REx™ cell lines

The T-REx™-293, T-REx™-HeLa, T-REx™-CHO, and T-REx™-Jurkat cell lines stably express the Tet repressor from the pcDNA™6/TR plasmid and should be maintained in medium containing blasticidin (see page 6). For more information about pcDNA™6/TR, refer to the T-REx™ System manual or the pcDNA™6/TR manual. The T-REx™ System and pcDNA™6/TR manuals are available at **www.lifetechnologies.com** or by contacting Technical Support (see page 17).

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Media for cell lines

The table below provides the recommended complete medium, freezing medium, and antibiotic concentration required to maintain and culture each T-REx™ cell line. Cell culture reagents and blasticidin are available from Life Technologies (see page 15 for ordering information).

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

*FBS = fetal bovine serum

Important guidelines

- FBS does not need to be heat inactivated for use with these cell lines.
- Cell lines should be maintained in medium containing blasticidin at the concentrations listed above.
- If adherent cells (e.g., T-REx™-293, T-REx™-CHO, or T-REx™-HeLa) are split at a 1:5 to 1:10 dilution, they will generally reach 80–90% confluence in 3–4 days.

Methods

Culture T-REx™ cell lines

General cell handling

Follow the guidelines below to successfully grow and maintain your cells.

- **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 from Life Technologies, 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.

Tetracycline-reduced serum

When culturing T-REx™ cells, note that many lots of fetal bovine serum (FBS) may contain tetracycline as 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 may wish to culture the T-REx™ cell line in tetracycline-reduced FBS. For more information, consult the supplier of your serum.

Before starting

- 15-mL sterile, conical tubes
- 5-, 10-, and 25-mL sterile pipettes
- Cryovials
- Phosphate-Buffered Saline (PBS) (see **Recipes**, page 14)
- 0.4% Trypan blue in PBS and hemacytometer for counting cells
- Reagents to prepare the appropriate complete medium (see page 6)
- Freezing Medium (see pages 6 and 10)
- Table-top centrifuge
- 75-cm^2 flasks, 175-cm^2 flasks and other appropriately-sized flasks or plates
- Trypsin-EDTA solution or other trypsin solution

Continued on next page

Thaw adherent cells

The following protocol is designed to help you thaw adherent cells to initiate cell culture. All cell lines are supplied in vials containing 3×10^6 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 (at the recommended concentration listed on page 6).
7. Incubate the cells and check them daily until the cells are 80–90% confluent (2–7 days).
8. Proceed to **Passage adherent cells,** page 9.

Thaw suspension cells

The following protocol is designed to help you thaw suspension cells to initiate cell culture. All cell lines are supplied in vials containing 3×10^6 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 (at the recommended concentration listed on page 6).
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 9.

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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 page 6).

   Note: If you want the cells to reach confluency sooner, split the cells at a lower dilution (i.e. 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 Steps 1–8 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-cm^2 flasks, transfer 1 mL of the 10 mL cell suspension from Step 3 to a new 75-cm^2 flask and add 15 mL of fresh, complete medium containing the appropriate concentration of antibiotic (see page 6).

   Note: You may split the cells at a lower dilution (i.e. 1:4).
5. 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.
6. Incubate flasks in a humidified, 37°C, CO_2 incubator.

Repeat Steps 1–6 as necessary to maintain or expand cells.

Freeze cells

Introduction

When freezing the T-REx™ cell lines, we recommend:

- Freezing cells at a density of **at least** 3×10^6 cells/mL.
- Using a freezing medium composed of 45% complete medium, 45% conditioned complete medium, and 10% DMSO.

Obtain conditioned complete medium

To prepare freezing medium, you will need to obtain conditioned complete medium. Conditioned medium is the medium in which cells have been growing. To obtain conditioned medium, you may:

- Harvest the medium from the cells one day before you plan to freeze them by performing the steps in the table below.
- Harvest the medium from the cells just prior to freezing. Follow the procedure below 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:

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.

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Freeze cells, continued

Freeze cells

Before starting, label cryovials and prepare freezing medium (see page 10). Keep the freezing medium on ice.

1. To collect cells, perform the following:
   - For adherent cells, follow Steps 1–5 of **Passage adherent cells**, page 9. 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 and carefully aspirate off the medium.
4. Resuspend the cells at a density of **at least** 3×10^6 cells/mL in chilled freezing medium (see page 10).
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 for long-term storage.

Transfection

Transfection methods

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 (Chen and Okayama, 1987; Wigler et al., 1977), lipid-mediated transfection (Felgner et al., 1989; Felgner and Ringold, 1989), and electroporation (Chu et al., 1987; Shigekawa and Dower, 1988). We typically use lipid-mediated transfection to introduce the T-REx™ expression construct containing your gene of interest into T-REx™ cell lines. The table below lists the recommended transfection reagent for each T-REx™ cell line. Other transfection reagents may be suitable.

To transfect...	Use...
T-REx™-293 T-REx™-HeLa T-REx™-CHO	Lipofectamine® 2000 Reagent
T-REx™-Jurkat	DMRIE-C

Both Lipofectamin® 2000 and DMRIE-C are available from Life Technologies (see page 15 for ordering information). **Note:** Other transfection reagents including the Calcium Phosphate Transfection Kit are also available from Life Technologies. For more information, see **www.lifetechnologies.com** or contact Technical Support (see page 17).

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 (see above).
- 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.

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Generate double 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™5/TO, or pT-REx-DEST-based construct and dual selection with either Zeocin™ (for pcDNA™4/TO-based constructs), hygromycin (for pcDNA™5/TO-based constructs), or Geneticin® (for pT-REx-DEST-based constructs) and blasticidin. **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. Zeocin™, hygromycin, and Geneticin® antibiotics may be obtained from Life Technologies (see page 15 for ordering information).

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

Appendix A: Supplemental information

Recipes

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 g KCl
   1.44 g Na_2HPO_4
   0.24 g KH_2PO_4

2. Adjust pH to 7.4 with concentrated HCl.
3. Bring the volume to 1 liter and autoclave for 20 minutes on liquid cycle.
4. Store at 4°C or room temperature.

Appendix B: Ordering information

Accessory 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 of pcDNA™4/TO, pcDNA™5/TO, and pT-REx™-DEST-based constructs, respectively, after transfection into T-REx™ cell lines. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 17).

Item	Amount	Cat. no.
Blasticidin	50 mg	R210-01
Zeocin™ Selection Antibiotic	8 x 1.25 mL	R250-01
	50 mL	R250-05
Hygromycin-B	20 mL	10687-010
Geneticin® Selective Antibiotic	20 mL	10131-035
Lipofectamine® 2000 Reagent	0.75 mL	11668-027
	1.5 mL	11668-019
DMRIE-C	1 mL	10459-014

Cell culture reagents

A large variety of Gibco® cell culture products are available from Life Technologies to facilitate growth and maintenance of the T-REx™ cell lines. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 17).

Note: Reagents are available in other sizes.

Item	Amount	Cat. no.
Dulbecco's Modified Eagle Medium (D-MEM)	500 mL	11965-092
Minimum Essential Medium with Earle's Salts (E-MEM)	500 mL	10370-021
Ham's F-12	500 mL	11765-054
RPMI 1640 Medium	500 mL	11875-093
200 mM L-Glutamine	100 mL	25030-081
Penicillin-Streptomycin	100 mL	15070-063
Trypsin-EDTA	100 mL	25300-054

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Accessory products, continued

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. For more information, refer to **www.lifetechnologies.com** or contact Technical Support (see page 17).

Item	Amount	Cat. no.
pcDNA™4/TO	20 µg	V1020-20
pcDNA™4/TO/*myc*-His A, B, & C	20 µg each	V1030-20
pcDNA™5/TO	20 µg	V1033-20
Gateway® pTREx-DEST30 Vector	6 µg	12301-016
Gateway® pT-REx-DEST31 Vector	6 µg	12302-014

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. The table below lists Flp-In™ T-REx™ products available from Life Technologies. For more information about the Flp-In™ T-REx™ System and generating Flp-In™ T-REx™ host cell lines, refer to **www.lifetechnologies.com** or contact Technical Support (see page 17).

Item	Amount	Cat. no.
pFRT/*lac*Zeo	20 µg	V6015-20
pFRT/*lac*Zeo2	20 µg	V6022-20
pcDNA™5/FRT/TO	20 µg	V6520-20
pcDNA™5/FRT/TO TOPO TA Expression Kit	20 reactions	K6510-20
pOG44	20 µg	V6005-20

Documentation and Support

Obtaining support

Technical Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

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

Safety Data Sheets (SDS)

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

Limited product warranty

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