Jump-In™ T-Rex™ CHO-K1 Retargeting Kit

Catalog Number A15007

Doc. Part No. A15007PIS **Pub. No.** MAN0007254 **Rev.** A.0

Product description

The Jump-In™ T-REx™ CHO-K1 Retargeting Kit allows the targeted integration of your gene of interest into a specific pre-engineered R4 site in Jump-In™ T-REx™ CHO-K1 cells to create an inducible isogenic stable cell line. These cells stably express the tetracycline repressor protein, allowing for inducible expression of your retargeted gene of interest upon the addition of doxycycline. The Jump-In™ T-REx™ CHO-K1 Retargeting Kit allows for inducible expression of your toxic or unstable gene of interest and is the ideal solution for cells and assays where transient engineering technologies are problematic, as well as for difficult to engineer cell lines. The kit also provides a convenient way to create target panels of gene families, isoforms, or orthologs.

Component	Amount/Composition	Storage
Jump-In™ T-REx™ CHO-K1 Cells	2 vials (~3 × 10^6 cells/vial in Freezing Medium*)	Liquid nitrogen
pJTI™ R4 Int (integrase vector)	100 µg at 1.5 µg/µL in TE buffer, pH 8.0**	–20°C
pJTI™ R4 DEST CMV-TO pA (destination vector)	100 µg at 1.5 µg/µL in TE buffer, pH 8.0	–20°C

* Recovery™ Cell Culture Freezing Medium; **TE buffer, pH 8.0: 10 mM Tris-HCl, 1 mM EDTA, pH 8.0

Guidelines for use

- For additional materials required but not provided, go to **www.thermofisher.com** and search for A15007 to download the Jump-In™ T-REx™ CHO-K1 Retargeting Kit user guide (Pub. No. MAN0007277), which provides the full detailed protocol. For first-time users, we recommend following the detailed protocol available online.
- After the initial thaw and passage, Jump-In™ T-REx™ CHO-K1 cells usually double in about 24 hours.
- **We highly recommend** that you include the pJTI™ R4 EXP CMV-TO EmGFP pA vector (Cat. No. A15005) in your Jump-In™ T-REx™ retargeting experiment as a positive control along with negative controls (no plasmid DNA, no integrase vector) to visualize the results and optimize the retargeting conditions.

Experiment outline

The following table describes the major steps required to retarget the Jump-In™ T-REx™ CHO-K1 cell line.

Table 1 Retargeting experiment workflow

Step	Action
1	Thaw and expand the Jump-In™ T-REx™ CHO-K1 cells
2	Create an entry clone by cloning your gene of interest into a Gateway™ entry vector
3	Generate a retargeting construct by performing an LR recombination reaction between the entry clone and pJTI™ R4 DEST CMV-TO pA (i.e., the destination vector)*
4	Co-transfect your retargeting construct and the integrase vector into the Jump-In™ T-REx™ CHO-K1 cells
5	Select for retargeted Jump-In™ T-REx™ CHO-K1 cells in Selection medium containing Geneticin™.
6	Confirm the retargeting of the Jump-In™ T-REx™ CHO-K1 cells by PCR
7	Characterize the retargeted clones

* Alternatively, you can clone your gene of interest into pJTI™ R4 CMV-TO MCS pA vector (Cat. No. A15004) via restriction enzyme cloning.

IMPORTANT! This product information sheet offers instructions and guidelines for thawing and propagating Jump-In™ T-REx™ CHO-K1 cells, and provides only an overview of retargeting experiments. For detailed instructions for creating a retargeting construct, transfecting (retargeting) the Jump-In™ T-REx™ CHO-K1 cell line, and selecting and characterizing the retargeted clones, refer to the Jump-In™ T-REx™ CHO-K1 Retargeting Kit user guide (Pub. No. MAN0007277), which provides the full detailed protocol.

Jump-In™ T-REx™ CHO-K1 cell culture

Table 2 Media used in culturing Jump-In™ T-REx™ CHO-K1 cells

Component	Thawing medium	Growth medium	Retargeting selection medium	Catalog No.
D-MEM with GlutaMAX™-I (high glucose)	90%	90%	90%	10569010
Dialyzed FBS *(Do not substitute!)*	10%	10%	10%	26400036
MEM Non-Essential Amino Acids Solution	0.1 mM	0.1 mM	0.1 mM	11140050
HEPES Buffer (pH 7.3)	25 mM	25 mM	25 mM	15630080
Penicillin(antibiotic)	100 U/mL	100 U/mL	100 U/mL	15140122
Streptomycin (antibiotic)	100 µg/mL	100 µg/mL	100 µg/mL	15140122
Geneticin™	—	—	5 mg/mL	11811031
Hygromycin B	—	200 µg/mL	—	10687010
Blasticidin	—	—	10 µg/mL	A1113902

Thaw Jump-In™ T-REx™ CHO-K1 cells

1. Rapidly thaw the cells with gentle agitation in a 37°C water bath.
2. Exchange media by transferring the thawed cells into 10 mL of Thawing medium in a sterile 15-mL tube, centrifuge at 200 × *g* for 5 minutes, then resuspend the cells in 1 mL of fresh Thawing medium.
3. Transfer the cells to a T-75 tissue culture flask containing 20 mL of pre-equilibrated Thawing medium, then place the flask in a humidified 37°C/5% CO_2 incubator.
4. At first passage, switch to Growth medium.

Propagate Jump-In™ T-REx™ CHO-K1 cells

1. Aspirate medium from growing cells, rinse once in PBS, then add the appropriate amount of 0.05% Trypsin/EDTA (3 mL for a 100-mm dish, 5 mL for a T-75 flask, 10 mL for a T-175 flask).
2. Add an equal volume of Growth medium to inactivate the 0.05% Trypsin/EDTA.
3. Verify under a microscope that cells have detached and clumps have completely dispersed.
4. Determine the viable cell number using a hemocytometer or a cell counter. Cell number and viability can be quickly and conveniently determined using the Countess™ II Automated Cell Counter. We recommend determining cell health frequently to ensure optimal performance in retargeting experiments.
5. Centrifuge the cells at 200 × *g* for 5 minutes, then resuspend them in Growth medium.
6. Seed fresh culture vessel containing pre-warmed Growth medium at the appropriate cell density. We recommend a split ratio of 1:3 to 1:10.

   IMPORTANT! Do **not** allow the cells to reach confluence.

Technical Support

For assistance, contact our Technical Support team at **drugdiscoverytech@thermofisher.com** or 760-603-7200 (enter 3 for "know your party's extension", then enter 40266).

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 found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

The performance of this product is guaranteed for six months from the date of purchase if stored and handled properly.

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

DISCLAIMER

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important Licensing Information: This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Manufacturer: Life Technologies Corporation | 5791 Van Allen Way | Carlsbad, CA 92008

thermofisher.com

20 June 2017

ThermoFisher SCIENTIFIC