invitrogen PRODUCT INFORMATION SHEET

Jump-In™ CHO-K1 Retargeting Kit

Catalog Number A14148

Doc. Part No. A14148PIS **Pub. No.** MAN0005285 **Rev.** A.0

Product description

The Jump-In™ CHO-K1 Retargeting Kit allows the targeted integration of your gene of interest into a specific pre-engineered R4 site in Jump-In™ CHO-K1 cells to create an isogenic stable cell line. The Jump-In™ CHO-K1 Retargeting Kit is the ideal solution for cells and assays where transient engineering technologies are problematic, as well as for difficult to engineer cell lines. The kitalso provides a convenient way to create target panels of gene families, isoforms, or orthologs.

Component	Amount/Composition	Storage
Jump-In™ 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 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 A14148 to download the Jump-In™ CHO-K1 Retargeting Kit user guide (Pub. No. MAN0005450), 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™ CHO-K1 cells usually double in about 30 hours.
- **We highly recommend that** you include all the suggested negative and positive controls in your transfection (retargeting) experiments. The controls provide a good indicator for the principle success of the retargeting reaction and are crucial for any troubleshooting.
- **Positive control:** pJTI™ R4 EXP CMV EmGFP pA (positive control vector, Cat. No. A14146) + pJTI™ R4 Int (integrase vector, provided in this kit).

Experiment outline

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

Table 1 Retargeting experiment workflow

Step	Action
1	Thaw and expand the Jump-In™ 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 pA (i.e., the destination vector)
4	Co-transfect your retargeting construct and the integrase vector into the Jump-In™ CHO-K1 cells
5	Select for retargeted Jump-In™ CHO-K1 cells in Selection medium containing Blasticidin
6	Confirm the retargeting of the Jump-In™ CHO-K1 cells by PCR
7	Characterize the retargeted clones

IMPORTANT! This product information sheet offers instructions and guidelines for thawing and propagating Jump-In™ CHO-K1 cells, and provides only an overview of retargeting experiments. For detailed instructions for creating a retargeting construct, transfecting (retargeting) the Jump-In™ CHO-K1 cell line, and selecting and characterizing the retargeted clones, refer to the Jump-In™ CHO-K1 Retargeting Kit user guide (Pub. No. MAN0005450), which provides the full detailed protocol.

Jump-In™ CHO-K1 cell culture

Table 2 Media used in culturing Jump-In™ 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
Hygromycin B	—	200 µg/mL	—	10687010
Blasticidin	—	—	10 µg/mL	A1113902

Thaw Jump-In™ 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™ 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).

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