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Product Information Sheet

Jump-In™ CHO-K1 Retargeting Kit

Catalog Number: A14148 | **Literature Part Number: A14148PIS** (MAN0005285)
Literature Lot Number: V01.00 | Revision date: 5 October 2011

Kit components	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)	200 µL at 0.5 µg/µL in TE buffer, pH 8.0**	–20°C
pJTI™ R4 DEST CMV pA (destination vector)	200 µL at 0.5 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

FAST FACTS

- For additional materials required but not provided, visit www.lifetechnologies.com and search for A14148 to download 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 table below describes the major steps required for retargeting 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	Cotransfect 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 retargeting of the Jump-In™ CHO-K1 cells by PCR
7	Characterize the retargeted clones

Note that 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 full detailed protocol provided online.

Technical Support

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

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 number
D-MEM with GlutaMAX™-I (high glucose)	90%	90%	90%	10569-010
Dialyzed FBS *(Do not substitute!)*	10%	10%	10%	26400-036
MEM Non-Essential Amino Acids Solution	0.1 mM	0.1 mM	0.1 mM	11140-050
HEPES Buffer (pH 7.3)	25 mM	25 mM	25 mM	15630-080
Penicillin(antibiotic)	100 U/mL	100 U/mL	100 U/mL	15140-122
Streptomycin (antibiotic)	100 µg/mL	100 µg/mL	100 µg/mL	15140-122
Hygromycin B	—	200 µg/mL	—	10687-010
Blasticidin	—	—	10 µg/mL	A11139-02

Thawing 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, and 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 and place the flask in a humidified 37°C/5% CO_2 incubator.
4. At first passage, switch to Growth Medium.

Propagating Jump-In™ CHO-K1 Cells

1. Aspirate medium from growing cells, rinse once in PBS, and 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® 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 and resuspend then 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.

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