Applied StemCell, Inc.
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Datasheet

TARGATT™ CHO-K1 (H11) Bioproduction Kit

Product Information

Catalog Number

AST-1420

Description

The TARGATT™ CHO-K1 Bioproduction Kit was designed for fast and site-specific knock-in in Chinese Hamster Ovary (CHO) cells with an easy-to-use approach. The master cell line provided in this kit contains both the “attP” docking-site and the integrase expression cassette engineered into the CHO H11 safe harbor locus in the genome. Any gene of interest can be cloned into the provided TARGATT™ “attB” cloning plasmid and transfected into the master cell line for generating a stable knock-in cell line. The TARGATT™ integrase-based technology enables efficient DNA integration and high-level gene expression without disrupting internal genes. The TARGATT™ CHO-K1 cell line can therefore be used for uniform, site-specific gene knock-in, generation of isogenic cell lines, and amplification strategies for isolating high expression cells, without the need for single cell cloning.

The TARGATT™ CHO-K1 master cell line and transgenic kit are suitable for research applications involving gene overexpression and high-level expression of recombinant proteins and other biologics in a rapidly expanding bioproduction industry and for other applications*.

**TARGATT™ master cell lines can be generated in any cell line including stem cells. Please contact Applied StemCell for TARGATT™ cell line engineering services to generate a master cell line in a specific cell line of choice.*

Advantages of using TARGATT™ Master Cell Lines for gene knock-in:

- High efficiency, unidirectional integration
- Site-specific, stable knock-in cell line generation
- Single copy gene integration into safe harbor locus
- Gene expression from an active, intergenic locus
- Easy-to-use protocol

Parental Cell Line

CHO-K1 Suspension Cells

Contents

The TARGATT™ CHO-K1 (H11) Bioproduction Kit contains the following:

- TARGATT™ CHO-K1 Master Cell line (AST-1400-1)
- TARGATT™ 42 Dual CMV-Hyg-TK Cloning Plasmid (AST-3082)
- TARGATT™ 30 (CAG-GFP-TK-No hyg) (AST-3076)
- TARGATT™ CMV-Integrase Plasmid (AST-3071)

Shipping

Dry ice

Storage and Stability

Store TARGATT™ master cell line in liquid nitrogen freezer immediately upon receipt. Store the plasmids at -20°C. Do not freeze-thaw plasmids repeatedly. The products provided in this kit are stable for at least 6 months from the date of receiving when stored as directed.

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Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protective Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

This product is for research use only and not intended for human or animal diagnostic or therapeutic uses.

Schematic Representation of TARGATT™ Knockin Strategy

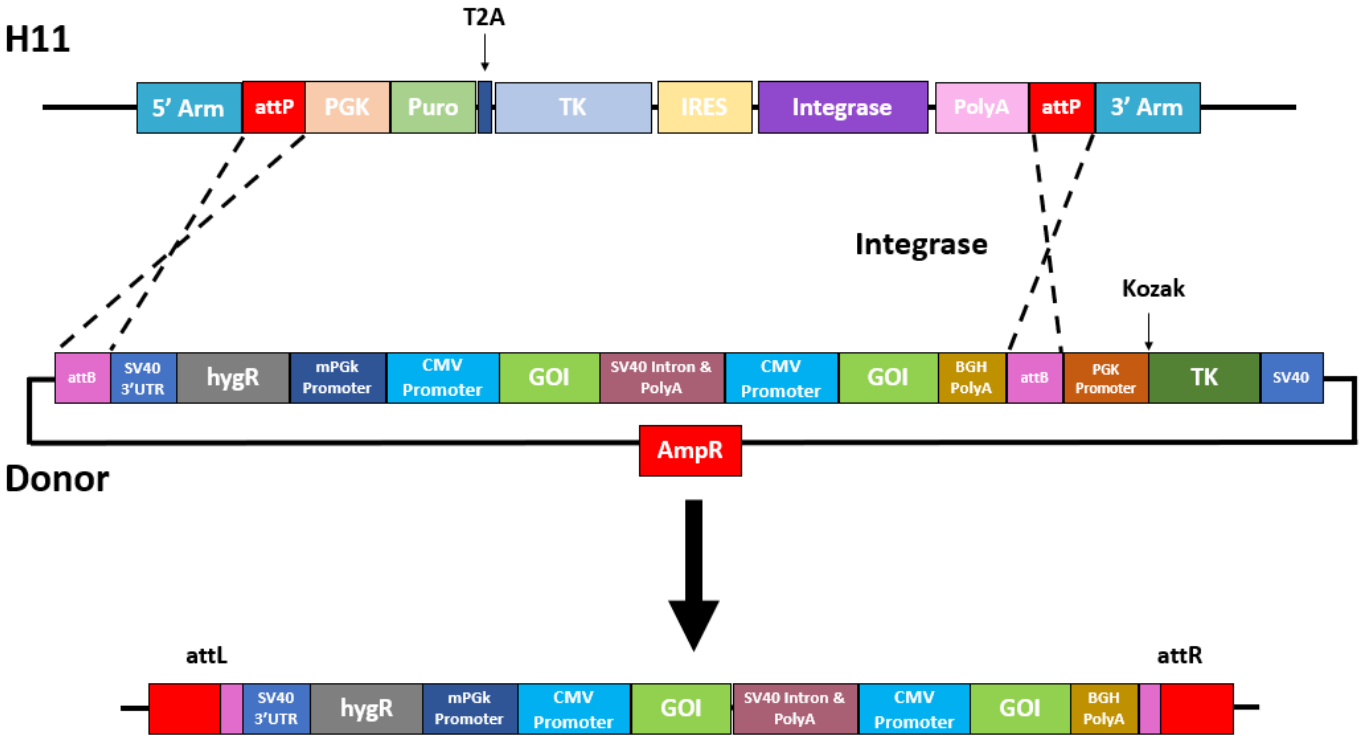

Figure 1. Schematic representation of TARGATT™ site-specific transgene integration mediated by integrase in TARGATT™ CHO-K1 (H11) master cell line. The TARGATT™CHO-K1 (H11) Master Cell Line was engineered with the attP landing pad at the Hipp11 (H11) safe harbor locus. The TARGATT™ plasmid containing the integrase recognition site, attB is used to clone the transgenes. The integrase catalyzes an irreversible reaction between the attP site in the genome and attB site in the donor vector, resulting in site-specific integration of the gene of interest at the selected locus. The cells that have stably knocked-in the donor plasmid can be enriched with hygromycin. Parental cells, random integrants and other undesired cells can be further eliminated via ganciclovir selection.

Media and Material

Catalog#	Component	Amount
AST-1400-1	TARGATT™ CHO-K1 (H11) Master Cell Line	2 x 10^6 cells
AST-3082	TARGATT™ 42 Dual CMV-Hyg-TK Cloning Plasmid	15 µg
AST-3076	TARGATT™ 30 (CAG-GFP-TK-No hyg)	8 µg
AST-3071	TARGATT™ CMV-Integrase Plasmid	15 µg

Materials Needed but not Provided

Material	Vendor	Cat. Number
BalanCD® CHO Growth A Medium	Irvine Scientific	91128
GlutaMAX™ Supplement	ThermoFisher	35050061
Dimethyl Sulfoxide (DMSO)	Sigma	D2438
Ganciclovir, 10mM/mL in DMSO	Selleck Chemicals	S1878
Hygromycin B Gold	InvivoGen	ant-hg-1: 10 x 1 ml (1 g)
NEB® 10-beta Competent E. coli (High Efficency)	New England BioLabs	C3019H
Corning® CoolCell® LX, Cell Freezing	Corning	432000
Neon™ Transfection System	ThermoFisher	MPK5000

Protocol

1. Preparation of Medium

CHO Culture Medium (Complete Culture Medium)

- BalanCD® CHO Growth A Medium
- GlutaMAX (8 mM)

Cryopreservation/ Freezing Medium

- CHO Culture Medium (90%)
- DMSO (10%)

2. Thawing and Culturing Cryopreserved CHO-K1 Cells

2.1. Warm up CHO culture medium at 37°C water bath. Before transferring into the hood, wipe the bottles with paper towel and again with paper towel sprayed with 70% ethanol to disinfect.

2.2. Aliquot 30 mL CHO culture medium into a 50 mL conical tube, and warm in a 37°C water bath for 10 minutes.

2.3. Retrieve a vial of frozen TARGATT™ CHO-K1 master cell line from the liquid nitrogen tank wearing a thermal glove.

2.4. Immediately immerse the vial in a 37°C water bath. While holding the top of the vial, gently agitate the vial. *Note: Gently agitate the vial to thaw the cells quickly; DO NOT keep it static while in the water bath; DO NOT submerge the cap.*

2.5. When almost completely thawed (usually takes ~1 minute, a small piece of ice is still visible), remove the vial from the water bath, and decontaminate the exterior of the vial with 70% ethanol. *Note: All further operations should be carried out under aseptic conditions.*

2.6. Transfer the contents of the vial to a conical tube containing 9.0 mL of pre-warmed complete culture medium.

2.7. Determine the viable cell count and calculate cell density, using an appropriate cell counter.

2.8. Centrifuge at **125 x g for 5 minutes.** Discard the supernatant.

2.9. Tap the tube to loosen up the cells and re-suspend the cell pellet in pre-warmed fresh complete culture medium at a density of 2.5 – 3.0 x10^5 viable cells/mL, in a fresh 125 mL Erlenmeyer shaker flask.

2.10. Place flask on an orbital shaker platform rotating at 130 rpm, in a 37°C and 5-8% CO2 incubator.

3. Passaging Procedure

3.1. Once the culture reaches 2.5 – 5.0 x10^6 cell/mL, the cells are ready to be passaged.

3.2. Warm-up complete culture medium in a 37°C water bath for 10 minutes.

3.3. Take the 125 mL Erlenmeyer shaker flask out of the CO_2 incubator.

3.4. Determine the viable and total cell counts.

3.5. Seed the cells at a density of 2.0 – 3.0 x10^5 viable cells/mL into pre-warmed fresh culture medium by gently pipetting. *Note: Calculate the volume of cell culture suspension and fresh complete medium needed to seed each new flask by dilution.*

3.6. Passage cells every 3-4 days.

3.7. Incubate cultures at 37°C and 5-8% CO_2.

4. Cryopreserving cells

4.1. Harvest cells and transfer the contents of the vial into a centrifuge tube.

4.2. Centrifuge at 125 x g for 5 minutes.

4.3. Aspirate the supernatant and resuspend the cells in cryopreservation medium (refer to step 1).

4.4. Aliquot 1 mL of the cell suspension into cryovials and transfer the vials into a CoolCell® cell freezing container and store the vials into a -80°C freezer overnight.

4.5. Ensure cells are completely frozen and transfer the cryovials into liquid nitrogen for long-term storage.

5. Transfection: Fast Knockin Procedure Using Neon™ Transfection System

Cloning with TARGATT™ 42 Dual CMV-Hyg-TK Cloning Plasmid (AST-3082)

5.1. Clone the protein of interest into the TARGATT™ 42 Dual CMV-Hyg-TK Cloning Plasmid (AST-3082) to make the donor plasmid using the single-step Golden-Gate cloning with PaqCI (AarI) method or the NheI-BglII/BamHi-SbfI restriction enzyme sites.
Note: See appendix for an example of how to design your cloning/library primers.

5.2. Transform the ligation or Golden Gate reaction into NEB® 10-beta Competent E. coli (High Efficiency) competent cells. Outgrow candidates, then mini-prep and screen them.
Note 1: E.coli that have taken in the GOI donor plasmid will be resistant to kanamycin, refer to the manufacturer's instructions for the selection concentration range that is acceptable.

5.3. Transform and midi-prep the desired clone. We recommend using a high-quality endo-free midi-prep kit for best results. Store at -20°C in a non-cycling freezer. Do not freeze-thaw the plasmid repeatedly.
Note 1: Do not use 5-alpha competent cells to transform the plasmids.

Preparation of Neon™ Transfection System

5.4. Aliquot a sufficient amount of Buffer E2 and Buffer R and warm at room temperature for at least 30 minutes.

5.5. Turn on the Neon™ Transfection System 30 minutes before use.

Cell Preparation for Electroporation

5.6. Pre-warm 6-well plates with 3 mL of fresh cell culture medium per well at 37°C.

5.7. Using cells already in the culture medium, count the cells and ensure >90% cell viability.

5.8. Harvest 1.1 x 10^6 cells and centrifuge the cells at 125 x g for 5 minutes.

5.9. Aspirate the supernatant and wash the cells by resuspending in 5 mL PBS.

5.10. Centrifuge the cell suspension at 125 x g for 5 minutes. Aspirate the supernatant.

5.11. Resuspend the cells by adding 110 µL Neon® Buffer R.

5.12. Aliquot a 110 µL cell suspension into a 1.5 mL Eppendorf tube and add 5 µg of DNA (for one EP) which has 1.25 µg TARGATT™ CMV-Integrase Plasmid (AST-3071) + 3.75 µg donor plasmid (or the positive control TARGATT™ GFP plasmid).
Note: For optimal results, the concentration of the donor plasmid should be in the 1000~1500 ng/ul range.

5.13. Flick the tube a few times to mix them well.

5.14. Electroporate the cells based on the following condition: 1550 V, 30 ms, 1 Pulse with Neon™ electroporation or any other transfection method-of-choice.
Note: If using other transfection methods, please optimize your protocol accordingly.

5.15. After electroporation, plate the cells in one-well of 6-well-plate, and transfer the plate into an incubator at 37°C with 5-8% CO_2.

5.16. Place the culture plate containing the cells into an incubator at 37°C with 5-8% CO_2, on an orbital shaker platform rotating at 130 rpm.

6. Hygromycin Selection (Enrichment) Procedure

6.1. On day 3 after electroporation, discard the spent medium, then adjust the cell concentration to 1 x 10^6 cells/mL in 3 mL complete culture medium with 400 µg/mL hygromycin per well.
Note: Hygromycin cannot be used with the GFP control.
Note 2: After the cells have been grown for 7-14 days, ganciclovir selection can be started if you would like to enrich for GFP knock-in cells from the positive control electroporation.

6.2. From day 4 to day 14, passage the cells if the concentration is >5 x 10^6 cells/mL, and always add 400 µg/mL hygromycin to the new medium. Keep hygromycin in the medium to enrich for the knocked-in cell population during this period.

7. Ganciclovir (GCV) Selection Procedure

6.3. On day 15 after electroporation, when cell viability is above 90%, adjust the cell concentration to 1 x 10^6 cells/mL using a total of 3 mL culture medium in a 6-well plate.

6.4. Add 1 µg/mL of ganciclovir into culture medium with 400 µg/mL hygromycin.

6.5. On day 3 of GCV selection, harvest the cells and centrifuge the cell suspension at 125 x g for 5 minutes.

6.6. Remove the supernatant and resuspend the cells in fresh culture medium with 1 µg/mL GCV and 400 µg/mL hygromycin.

6.7. Transfer the cells at a cell density of 1 x 10^6 cells/mL into a fresh 6-well culture plate.

6.8. Monitor the cell viability daily during GCV selection.

6.9. Stop GCV killing if the cell viability drops to ~30-40%, or after 7 days.

6.10. To stop the GCV selection, harvest the cells and centrifuge the cell suspension at 125 x g for 5 minutes. Remove the supernatant and add fresh culture medium without GCV but with hygromycin, and culture the cells in a 6-well plate until they fully recover.

Supporting Data

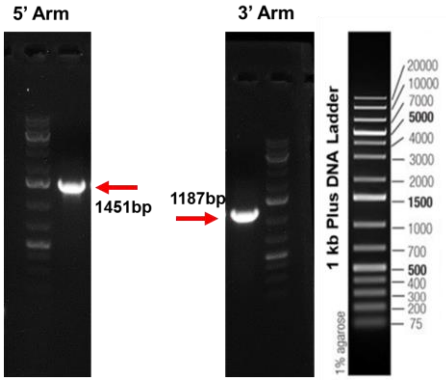

Figure 1. Genotyping of 5'-arm and 3'-arm in TARGATT™ CHO-K1 (H11) master cell line. PCR analysis shows the expected sizes for 5' arm and 3' arm junction PCR product in a 1.5 % agarose gel: 1451 bp and 1187 bp, respectively.

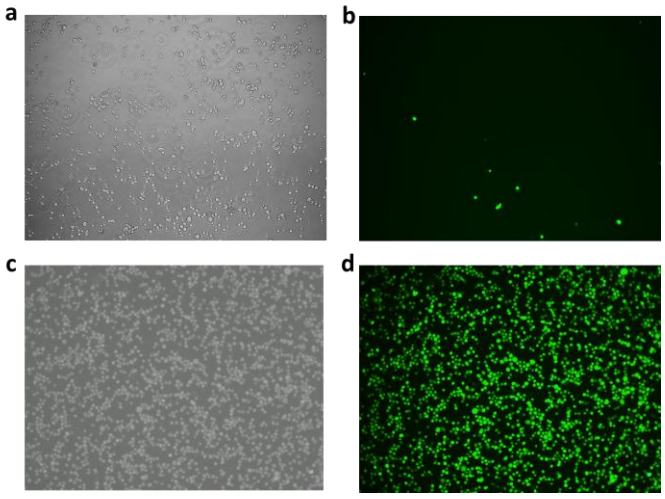

Figure 2. Uniform and Stable GFP expression 21 days post-transfection using TARGATT™ CHO-K1 (H11) Master Cell Line and Knockin Kit. The TARGATT™ CAG-GFP plasmid vector was used to evaluate gene integration in the parental CHO-K1 cells (a-b) and TARGATT™ CHO-K1 (H11) master cell line (c-d). An enriched GFP signal was detected by fluorescence imaging in TARGATT™ CHO-K1 (H11) Master Cell Line. (Left) bright field microscopy. (Right) Immunofluorescence; GFP channel.

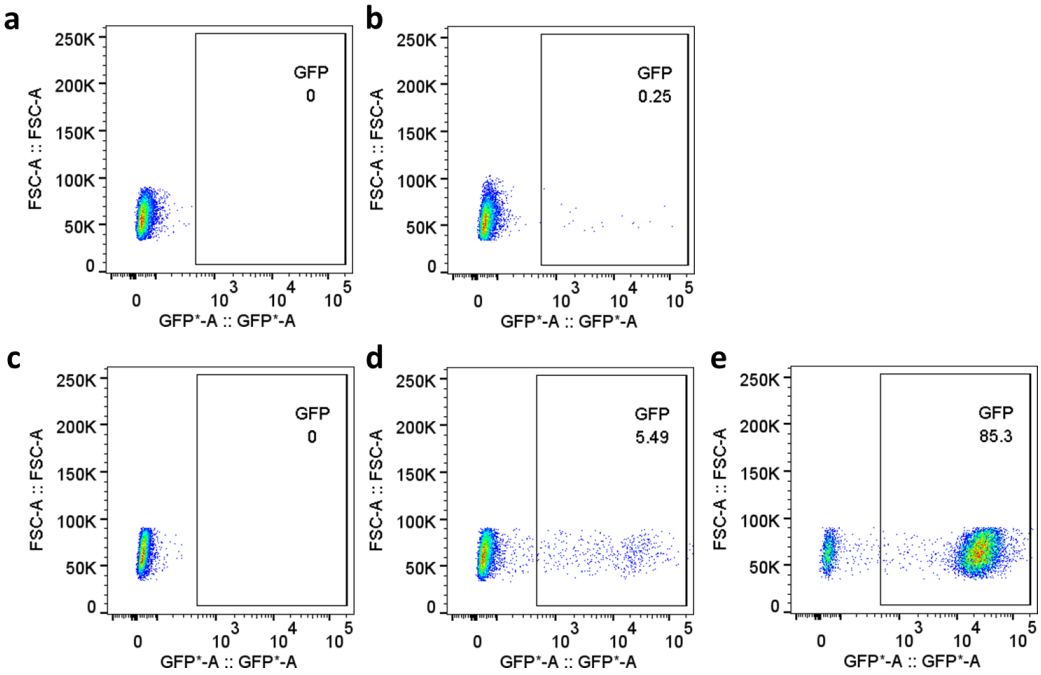

Figure 3. Flow cytometric analysis of GFP expression level in CHO-K1 cells. GFP expression was measured by flow cytometric after transfection of TARGATT™ CAG-GFP plasmid in parental CHO cell line by random integration (c-b) and in TARGATT™ CHO-K1 (H11) Master Cell Line by site-specific gene integration (c-e). (a) CHO-K1 parental cell line without transfection; (b) CHO-K1 parental cell line randomly transfected with TARGATT™ CAG-GFP plasmid; (c) TARGATT™ CHO-K1 (H11) master cell line without transfection; (d) TARGATT™ CHO-K1 (H11) master cell line transfected with TARGATT™ CAG-GFP plasmid before GCV selection; (e) TARGATT™ CHO-K1 (H11) master cell line transfected with TARGATT™ CAG-GFP plasmid after GCV selection.

Appendix

These primer examples are for PaqCI / AarI Golden Gate cloning, which require that your library not have its binding site. If necessary, alternative primers can be designed using other type IIS restriction enzymes. If you choose to use other restriction enzymes, please contact Applied StemCell for assistance.

ID	Primer	Sequence
1	Forward Primer for 1st Protein / Coding Sequence	5'-gttgtt CACCTGC tttt CCGC caccatg … -3'
2	Reverse Primer for 1st Protein / Coding Sequence	5'-caacaa CACCTGC aaaa GCAT cta … -3'
3	Forward Primer for 2nd Protein / Coding Sequence	5'- gttgtt CACCTGC tttt CACC atg … -3'
4	Reverse Primer for 2nd Protein / Coding Sequence	5'-caacaa CACCTGC aaaa TAGT cta … -3'

Primer IDs	Sequence	Explanation
1, 3	gttgtt	Six-base filler sequence to ensure restriction enzyme cleavage
1, 3	CACCTGC tttt	PaqCI / AarI recognition sequence and a 4 base filler
1	CCGC cacc	5' Golden Gate overhang for cloning 1st protein, and part of the Kozak sequence
3	CACC	5' Golden Gate overhang for cloning the 2nd protein
1, 3	atg …	Start codon and sequence that is specific to the protein of interest
2, 4	caacaa	Six-base filler sequence to ensure restriction enzyme cleavage
2, 4	CACCTGC aaaa	PaqCI / AarI recognition sequence and a 4 base filler
2	GCAT	Reverse complement of the 3' Golden Gate overhang for cloning the 1st protein
4	TAGT	Reverse complement of the 3' Golden Gate overhang for cloning the 2nd protein
2, 4	cta …	Reverse complement of the 'tag' stop codon, and sequence that is specific to the protein of interest