Applied StemCell, Inc.
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Datasheet

TARGATT™ CHO-K1 (A2) Master Cell Line & Knockin Kit

Product Information

Catalog Number

AST-1405

Description

The TARGATT™ CHO-K1 Master Cell Line and transgenic kit was designed for fast and site-specific knockin in Chinese Hamster Ovary (CHO) cells using an easy-to-use gene knockin approach. The master cell line provided in this kit contains both the "attP" docking-site and the PhiC31 integrase expression cassette engineered into the CHO ASC2 (A2) safe harbor locus in the genome. Any gene of interest can be cloned into the provided TARGATT™ "attB" cloning plasmid (under control of the strong CAG promoter or promoter-of-choice) and transfected into the master cell line for generating a stable knockin 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 knockin, 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 knockin:

- High efficiency, unidirectional integration
- Site-specific, stable knockin cell line generation
- Single copy gene integration into safe harbor locus
- Gene expression from an active, intergenic locus
- Easy-to-use protocol requiring transfection of only one (donor) plasmid

Parental Cell Line

CHO-K1 Suspension Cells

Contents

The TARGATT™ CHO-K1 (A2) Master Cell Line & Knockin Kit contains the following:

- TARGATT™ CHO-K1 (A2) Master Cell line (AST-1405-1)
- TARGATT™ 20.1 (CAG-MCS-TK) cloning plasmid (AST-3068)
- TARGATT™ 21 (CAG-GFP) positive control plasmid (AST-3061)

Shipping

Dry ice

Storage and Stability

Store TARGATT™ master cell line in liquid nitrogen freezer immediately upon receipt. Store the plasmids -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

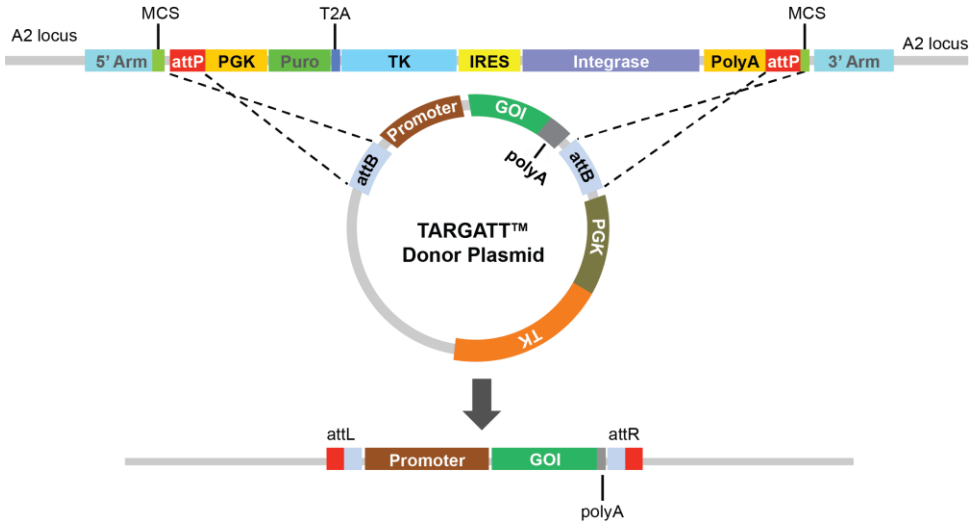

Figure 1. Schematic representation of TARGATT™ site-specific transgene integration mediated by integrase in TARGATT™ CHO-K1 master cell line. The TARGATT™CHO-K1 (A2) Master Cell Line was engineered with the attP landing pad at the proprietary ASC2 (A2) safe harbor locus. The TARGATT™ plasmid containing the integrase recognition site, attB is used to clone the transgene. 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 containing the gene of interest can be enriched using ganciclovir negative selection.

Media and Material

Catalog#	Component	Amount
AST-1405-1	TARGATT™ CHO-K1 (A2) Master Cell Line	2 x 10^6 cells
AST-3068	TARGATT™ 20.1 (CAG-MCS-TK) Cloning Plasmid	2 µg
AST-3061	TARGATT™ 21 (CAG-GFP-TK) Positive Control Plasmid*	2 µg

** The provided positive control TARGATT™ 21 (CAG-GFP-TK) plasmid should be amplified before further use; aliquot into single-use quantity and store at -20°C; Do not freeze thaw the plasmid repeatedly.*

Materials & Equipment Required but not Provided

Material	Vendor	Cat. Number
BalanCD® CHO Growth A Medium	Irvine Scientific	91128
L-Glutamine (200 mM)	ThermoFisher	25030081
PBS (10X), pH 7.4	ThermoFisher	70011
Ganciclovir, 10mM/mL in DMSO	Selleck Chemicals	S1878
Dimethyl Sulfoxide (DMSO)	Sigma	D2438
Neon™ Transfection System	ThermoFisher	MPK5000R
Corning® CoolCell® LX, Cell Freezing	Corning	432000

Protocol

1. Preparation of Medium

CHO Culture Medium (Complete Culture Medium)

- BalanCD® CHO Growth A Medium
- L-Glutamine (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.
2.1 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.
2.2 Transfer the contents of the vial to a conical tube containing 9.0 mL of pre-warmed complete culture medium.
2.3 Determine the viable cell count and calculate cell density, using an appropriate cell counter.
2.4 Centrifuge at 125 x g for 5 minutes. Discard the supernatant.
2.5 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.
2.6 Place flask on an orbital shaker platform rotating at 130 rpm, in a 37°C and 5-8% CO_2 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

Plasmid amplification and cloning with TARGATT™ 20.1 (CAG-MCS-TK) cloning plasmid

5.1 Amplify the plasmids provided using NEB® 10-beta Competent E. coli (High Efficiency) competent cells
Note: Do not use 5-alpha competent cells to transform the plasmids.
5.2 Aliquot into single-use quantity and store at -20°C. Do not freeze-thaw the plasmid repeatedly.
5.3 Clone the gene of interest into the TARGATT™ 20.1 (CAG-MCS-TK) Cloning Plasmid to make the donor plasmid.

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 2 µg cloned TARGATT™ donor plasmid containing your gene of interest (or the positive control TARGATT™ GFP plasmid).
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 (o)r 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 At day 2-3 after electroporation, discard the spent medium and add 3 mL fresh, complete culture medium per well.
5.17 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. Drug selection procedure

6.1 Seven (7) to 10 days after electroporation, adjust the cell concentration to 1 x 10^6 cells/mL using a total of 3 mL culture medium in a 6-well plate.
Note: Passage the cells if the concentration is >5 x 10^6 cells/mL
6.2 Add 1µg/mL drug selection reagent (Ganciclovir; GCV) into culture medium.
6.3 On day 3 of selection, harvest the cells and centrifuge the cell suspension at 125 x g for 5 minutes.
6.4 Remove the supernatant and resuspend the cells in fresh culture medium with GCV
6.5 Transfer the cells at a cell density of 1 x 10^6 cells/mL into a fresh 6-well culture plate.
6.6 Monitor the cell viability daily during GCV selection.
6.7 Stop GCV killing when the cell viability drops to ~30-40%, which usually takes 4-5 days.
6.8 To stop the GCV selection, harvest the cells and centrifuge the cell suspension at 125 x g for 5 minutes.

6.9 Remove the supernatant and add fresh culture medium without GCV, and culture the cells in a 6-well plate until they fully recover.

Supporting Data

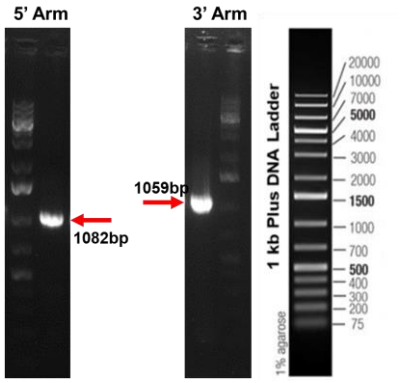

Figure 2. Genotyping of 5'-arm and 3'-arm in TARGATT™ CHO-K1 master cell line. PCR analysis shows the expected sizes for 5' arm and 3' arm junction PCR product in a 1.5 % agarose gel: 1082 bp and 1059 bp, respectively. Primer sequences used: 5'-arm-Forward: 5'-CTACCGGTGGATGTGGAATGTG-3'; 5'-arm-Reverse: 5'-AGTCCTGCGCACCTCGTTTTAG-3'; 3'-arm-Forward: 5'-CACCTCCCCCTGAACCTGAAAC-3'; 3'-arm-Reverse: 5'-CCCTGAAATTTCGATATCCACTGC-3'.

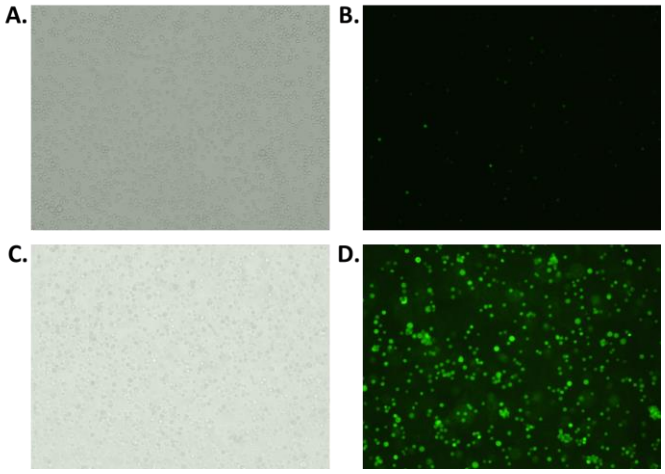

Figure 2. Uniform and Stable GFP expression 21 days post-transfection using TARGATT™ CHO-K1 (A2) Master Cell Line and Knockin Kit. The TARGATT™ CAG-GFP plasmid vector was used to evaluate gene integration in the parental CHO-K1 cells (a-b) and TARGATT™ CHO-K1 (A2) master cell line (c-d). An enriched GFP signal was detected by fluorescence imaging in TARGATT™ CHO-K1 (A2) Master Cell Line. (Left) bright field microscopy. (Right) Immunofluorescence; GFP channel.

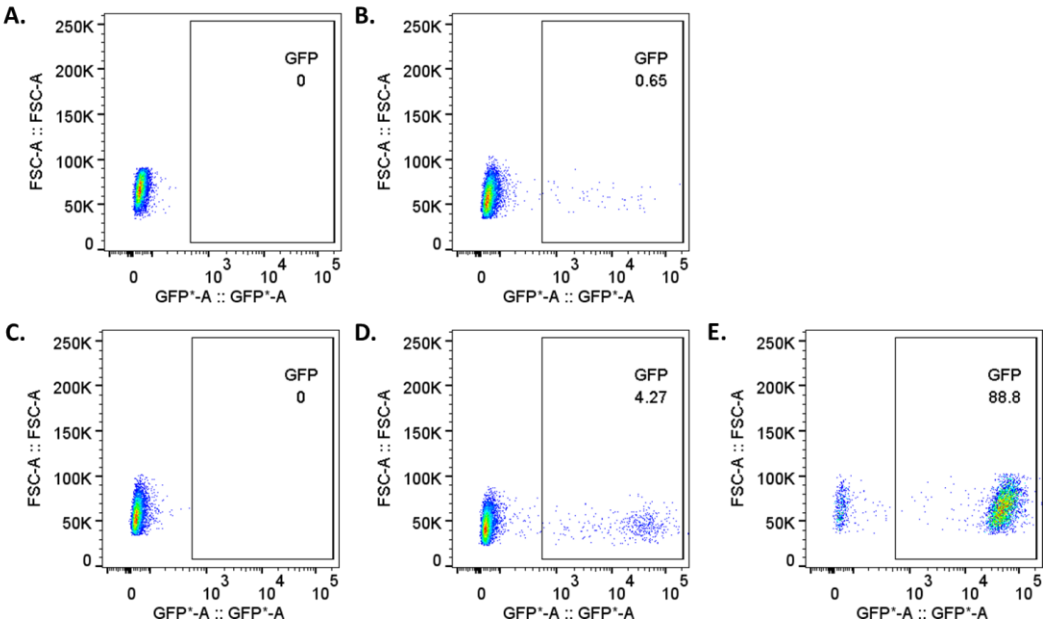

Figure 3. Flow cytometric analysis of GFP expression level in CHO-K1 cells. GFP expression was measured by flow cytometric after transfection of TARGATT™ CAG-GFP plasmid in parental CHO cell line by random integration (c-b) and in TARGATT™ CHO-K1 (A2) Master Cell Line by site-specific gene integration (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 (A2) master cell line without transfection; (d) TARGATT™ CHO-K1 (A2) master cell line transfected with TARGATT™ CAG-GFP plasmid before GCV selection; (e) TARGATT™ CHO-K1 (A2) master cell line transfected with TARGATT™ CAG-GFP plasmid after GCV selection.