Applied StemCell, Inc.
(866) 497-4180
www.appliedstemcell.com

Datasheet

# TARGATT™ CHO-K1 Master Cell Line & Knock-In Kit

## Product Information

**Catalog Number**

**AST-1200**

**Description**

The TARGATT™ CHO-K1 Master Cell Line and transgenic kit was designed for fast and site-specific knock-in in CHO cells using an easy-to-use gene knock-in approach. The master cell line provided in this kit contains both the "attP" docking-site and the PhiC31 integrase expression cassette engineered into the H11 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 knock-in cell line. The TARGATT™ integrase-based technology guarantees efficient DNA integration without bacterial backbone 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:**

- Stable cell line generation
- Single copy, site-specific gene integration into the safe harbor locus
- Guaranteed gene expression from an active, intergenic locus
- Footprint-free integration of gene of interest
- Use of selection marker is not necessary but optional
- Easy-to-use protocol requiring transfection of only one (donor) plasmid

**Parental Cell Line**

CHO-K1 Suspension Cells

**Contents**

All cell lines and reagents provided in this kit are sufficient for transfection according to the given protocol:

- TARGATT™ CHO-K1 Master Cell line (AST-1200-1)
- TARGATT™ 20 (CAG-MCS) cloning plasmid (AST-3060)
- TARGATT™ 21 (CAG-GFP) positive control plasmid (AST-3061)
- Ganciclovir

**Shipping**

Dry ice

**Storage and Stability**

Store TARGATT™ master cell line in liquid nitrogen freezer immediately upon receipt. Store the plasmids and ganciclovir at -20°C. Do not freeze-thaw plasmids

**Applied StemCell, Inc.**
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com

repeatedly. The products provided in this kit are stable for at least 6 months from the date of receiving when stored as directed.

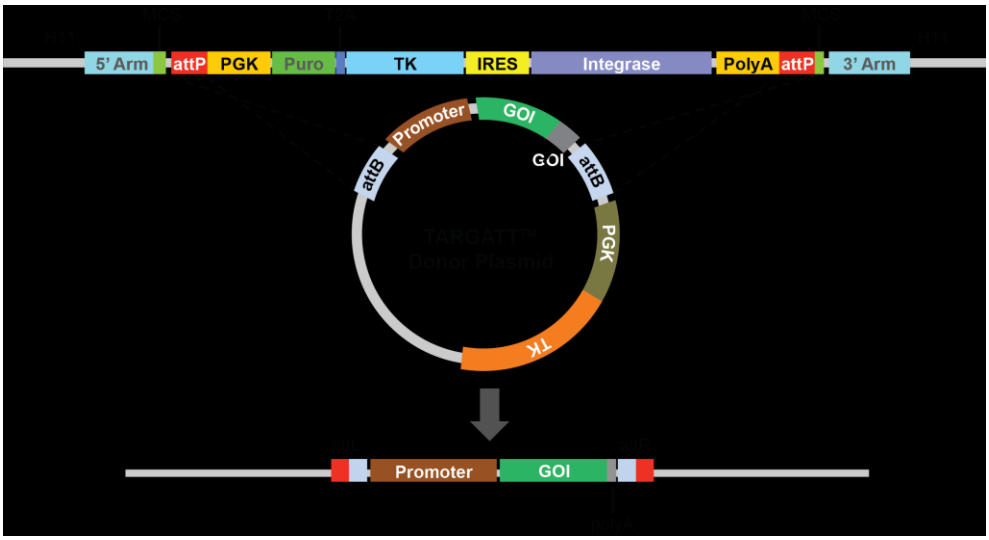
**Safety Precaution**

**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™ Knock-in Strategy




## Media and Material

| Catalog# | Component | Amount |
|---|---|---|
| AST-1200-1 | TARGATT™ CHO-K1 Master Cell Line | 1 x 10^6 cells |
| AST-1300-2 | 10X Ganciclovir | 15 µL |
| AST-3060 | TARGATT™ 20 (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) plasmid should be amplified before further use; aliquot into single-use quantity and store at -20°C; do not freeze thaw the plasmid repeatedly. We recommend using 2-5 µg of the positive control plasmid for each transfection.*

## Materials Needed but not Provided

| Material | Vendor | Cat. Number |
|---|---|---|
| BalanCD® CHO Growth A Medium | Irvine Scientific | 91128 |
| L-Glutamine, 200 mM | ThermoFisher | 25030081 |
| 100X Penicillin-Streptomycin (10,000 U/mL) | ThermoFisher | 15140163 |
| DMSO | Sigma | C6295 |

## Protocol

### 1. Preparation of Medium

**1.1 CHO Culture Medium**

- BalanCD® CHO Growth A Medium
- L-Glutamine Supplement (8 mM)
- Penicillin/ Streptomycin (1X); optional

**1.2 Freezing Medium**

- CHO Culture Medium
- DMSO (10%)

### 2. Thawing and culturing cryopreserved CHO-K1 cells

2.1 Quickly thaw (under 2minutes) the vial with gentle agitation in a 37°C water bath.
2.2 As soon as the contents are thawed, remove the vial from the water bath, and decontaminate the exterior of the vial with 70% ethanol. All further operations should be carried out under aseptic conditions.
2.3 Transfer the contents of the vial to a centrifuge tube containing 9.0 mL of complete culture medium, and centrifuge at approximately 125 x g for 5 minutes.
2.4 Re-suspend the cell pellet in 25 mL complete culture medium, and plate the suspension into a 125 ml Erlenmeyer shaker flask, on an orbital shaker platform rotating at 130 rpm.
2.5 Incubate the culture at 37°C and 5% $CO_2$.

### 3. Passaging procedure

3.1 Determine the viable and total cell counts.
3.2 Calculate the volume of cell culture suspension and fresh complete medium needed to seed each new flask by dilution.
3.3 Seed the cells at a density of 1.0 – 2.0 × 10^5 viable cells/mL with pre-warmed fresh culture medium by gently pipetting.
3.4 Passage cells every 3-4 days when they reach the density of approximately 1.0 – 2.0 x 10^6 viable cells/mL.
3.5 Incubate cultures at 37°C and 5% CO2.

### 4. Cryopreserving cells

4.1 Harvest cells and transfer the contents of the vial to a centrifuge tube
4.2 Centrifuge at approximately 125g for 5 minutes.
4.3 Aspirate the medium by glass Pasteur pipette.
4.4 Add freezing medium and resuspend the cells.
*Note: We suggest cryopreserving ~ 1.0 x 10^7 cells/mL freezing medium*

4.5 Aliquot the cells into cryovials.
4.6 Transfer the vials into a -80°C freezer using any of the cryopreservation containers.
4.7 After keeping the cells at -80C O/N or more than a day to ensure cells are completely frozen, transfer the cryovials into liquid nitrogen for long term storage.

### 5. Fast knock-in procedure

5.1 Pre-warm 6 well plates with 3mL fresh cell culture medium per well (without antibiotics) at 37°C.
5.2 Harvest cells and count cell number.
5.3 Transfer the contents of the vial to a centrifuge tube and centrifuge at approximately 125 x g for 5 minutes.
5.4 Aspirate the medium by glass Pasteur pipette.
5.5 Add 5 mL PBS and resuspend the cell pellet to wash. Spin down (125 x g for 5 minutes) and aspirate the PBS buffer.

5.6 Resuspend the cells by adding Neon® Buffer R to a cell density of 1 x 10^7/mL.
5.7 Aliquot 110 µL cell suspension in a 1.5 mL Eppendorf tube, add 2 µg cloned TARGATT™ donor plasmid (or the positive control TARGATT™ GFP plasmid). Flick the tube a few times to mix them well.
5.8 Electroporate the cells based on this condition: 1500 V, 30 ms, 1 Pulse with Neon electroporation or any other transfection method of choice.
5.9 After electroporation, plate the cells on one well of 6-well-plate and transfer the plate into an incubator. Culture the cells at 37°C/5% $CO_2$.

## 6. Drug selection procedure (optional)

6.1 Seven (7) days after electroporation, add 1µg/mL drug selection reagent (Ganciclovir) into culture medium. Keep the cells in the same culture condition.
6.2 Three (3) days after adding drug selection reagent, aspirate the drug medium and add fresh culture medium without selection reagent.

## Supporting Data

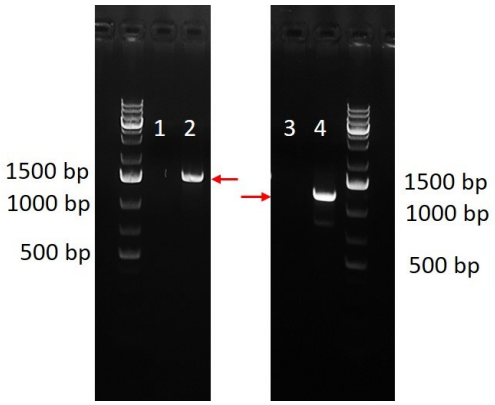



**Figure 1. Genotyping of 5'-arm and 3'-arm in TARGATT™ CHO-K1 master cell line.** RT-PCR analysis shows the expected sizes for 5' arm and 3' arm junction PCR product in a 1.5 % agarose gel. (1): CHO parental cell line with 5'-arm primers; (2): CHO master cells line with 5'-arm primers; (3): CHO parental cell line with 3'-arm primers; (4): CHO master cell line with 3'-arm primers.

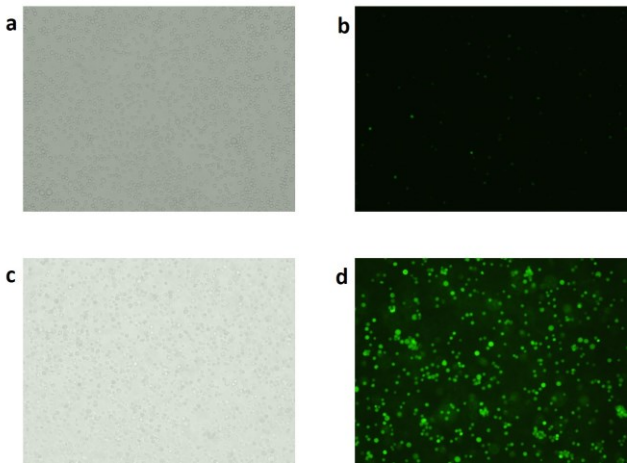



**Figure 2. Uniform and Stable GFP expression 21 days post-transfection**. The CAG-GFP vector was used to evaluate gene integration in the TARGATT™ CHO-K1 master cell line (c and d) and compared to random integration in parental CHO cell line (a and b). GFP signal was detected by fluorescence imaging. (Left) bright field microscopy. (Right) Immunofluorescence; GFP channel.

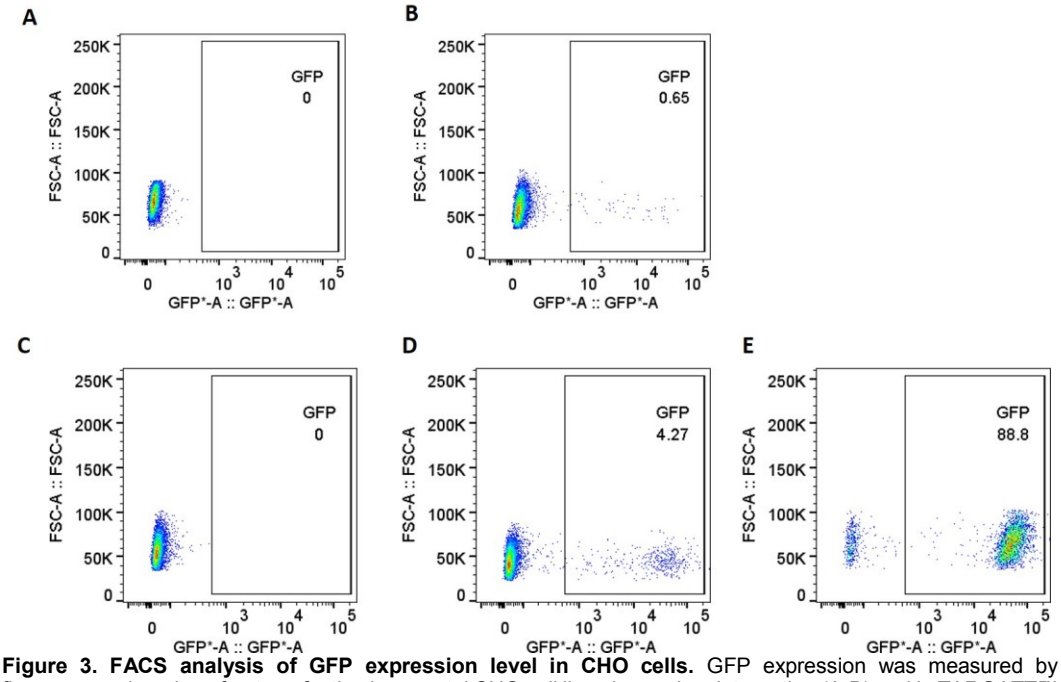



**Figure 3. FACS analysis of GFP expression level in CHO cells.** GFP expression was measured by fluorescence imaging after transfection in parental CHO cell lines by random integration (A-B) and in TARGATT™ CHO-K1 Master Cell Line by site-specific gene integration (C-E). (A) CHO parental cell line without transfection; (B) CHO parental cell line randomly transfected with GFP plasmid; (C) CHO master cell line without transfection; (D) CHO master cell line transfected with GFP plasmid before GCV selection; (E) CHO master cell line transfected with donor plasmid after GCV selection.