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Datasheet

TARGATT™ HEK293 High-Efficiency Sub-Line (HESL) Knock-in Kit (H11) for Library Screening (FACS)

Product Information

Catalog Number

AST-1308 TARGATT™ HEK293 High-Efficiency Sub-Line (HESL) Knock-in Kit (H11) for Library Screening (FACS)
Includes:
- **AST-1308-C** TARGATT™ HEK293 HESL Master Cell Line
- **AST-3101** TARGATT™ 54 attB-mCherry2-LZ Cloning Plasmid
- **AST-3102** TARGATT™ 55 pT1-mCherry2-HESL Positive Control Plasmid
- **AST-3201** TARGATT™ CMV-integrase Plasmid

Description

The TARGATT™ HEK293 HESL Knock-in Kit (H11) for Library Screening (FACS) is designed for fast, efficient, and site-specific integration of DNA fragments of up to 20 kb into HEK293 cells. The master cell line provided in this kit contains the TARGATT™ landing pad with an attP site engineered into the H11 safe harbor locus. The kit also contains a cloning plasmid containing a corresponding “attB” sequence into which any gene-of-interest can be cloned. The expression of the integrase (accomplished via the provided plasmid) mediates stable integration of the transgene into the master cell line (Figure 1). The TARGATT™ integrase enables highly efficient (>60% without enrichment; >90% with enrichment), and site-specific DNA integration without disruption of internal genes. The TARGATT HEK293 HESL Knock-in Kit (H11) for Library Screening (FACS) is ideal for single-transgene-per-cell knock-in and uniform, stable expression of your protein.

Advantages of using TARGATT™ Master Cell Lines for gene knock-in:
- High efficiency integration
- Site-specific, stable knock-in cell line generation
- Single copy gene integration into safe harbor locus
- Gene expression from an active, intergenic locus
- Low off-target integration

The TARGATT™ HEK293 HESL Knock-in Kit (H11) for Library Screening (FACS) includes the TARGATT™ pKHESL-mCherry-LZ (library) Cloning Plasmid which contains the fluorescent marker mCherry2. This kit is ideal for fluorescence activated cell sorting (FACS) and is suitable for research applications involving gene library construction.

**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 or to build your cell libraries.*

Parental Cell Line

HEK293 Adherent Cells, HESL variant

Contents

All cell lines and plasmids provided in this kit are sufficient for 27 transfections according to the given protocol:
- TARGATT™ HEK293 HESL Master Cell Line (AST-1308-C)
- TARGATT™ 54 attB-mCherry-LZ Cloning Plasmid (AST-3101)
- 55 pT1-mCh2-HESL Positive Control Plasmid (AST-3102)

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- TARGATT™ CMV-integrase Plasmid (AST-3201)

Shipping Dry ice

Storage and Stability Store the 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.

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

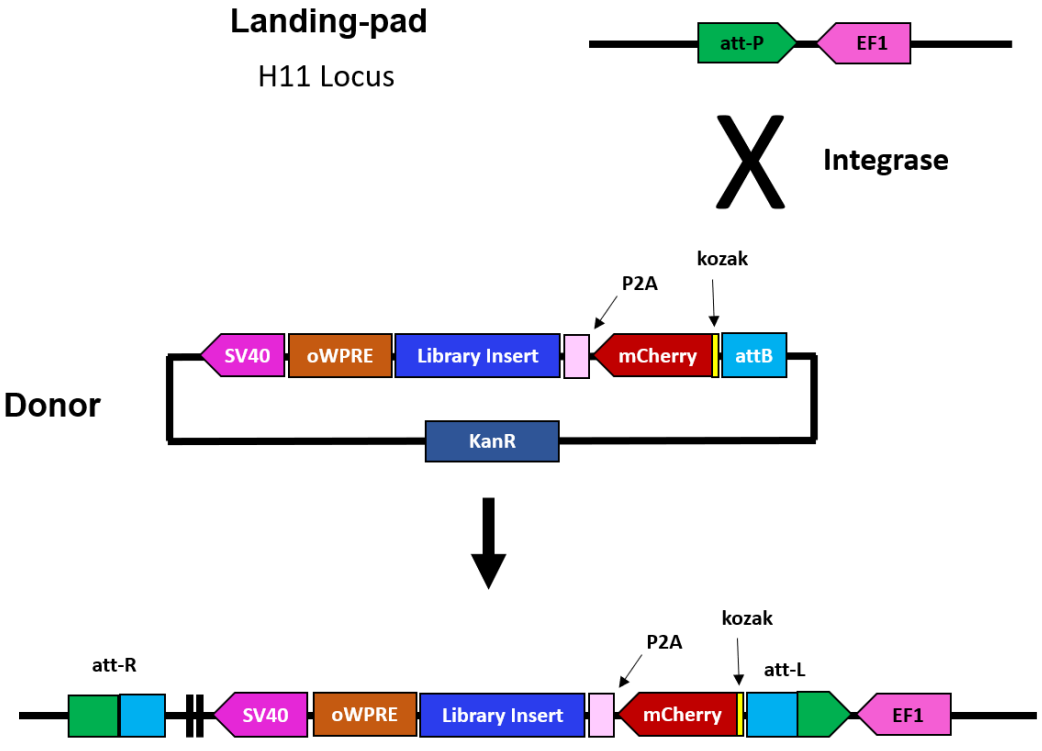

Figure 1. Schematic representation of TARGATT™ site-specific transgene integration mediated by the TARGATT™ integrase. The TARGATT™ HEK HESL Master Cell Line was engineered with the attP landing pad at the H11 safe harbor locus. The TARGATT™ plasmid containing the integrase recognition site, attB, is used to clone your transgene. The integrase catalyzes an irreversible reaction between the attP site in the genome and attB site in the donor vector, resulting in integration of your gene-of-interest at the H11 locus.

Media and Material

Catalog#	Component	Amount
AST-1308-C	TARGATT™ HEK293 HESL Master Cell Line	1 x 10^6 cells
AST-3101	TARGATT™ 54 attB-mCherry-LZ Cloning Plasmid[#]	15 µg
AST-3102	TARGATT™ 55 pT1-mCh2-HESL Positive Control Plasmid [#†]	15 µg
AST-3201	TARGATT™ CMV-integrase Plasmid[#†]	15 µg

[#] Aliquot into single-use quantities and store at -20°C; do not freeze-thaw the plasmid repeatedly. [†] The positive control and integrase plasmids are proprietary materials and should not be amplified. The provided quantity is sufficient for 27 transfections. Additional quantities of these plasmids are available for purchase.

Materials Needed but Not Provided

Material	Vendor	Catalog#
Dulbecco's Modified Eagle's Medium (DMEM), High Glucose, No Glutamine	ThermoFisher	11960077
Fetal Bovine Serum (FBS)	Biowest	S1620
100x Glutamax™ Supplement	ThermoFisher	35050061
100mM Sodium Pyruvate	ThermoFisher	11360070
1M HEPES	ThermoFisher	15630080
Gibco™ Penicillin-Streptomycin (10,000 U/mL)	ThermoFisher	15140148
Normocin™ - Antimicrobial Reagent, 500 mg	Invivogen	ant-nr-1
Xfect™ Transfection Reagent	Takara Bio	631318
DPBS, No Calcium, No Magnesium	ThermoFisher	14190136
Gibco™ TrypLE™ Express Enzyme (1X), phenol red	ThermoFisher	12605028
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	D2438
E. cloni ® 10G SUPREME Electrocompetent Cells (or comparable DH10B product)	Biosearch Tech	60080-1 60080-2 60081-1
Living Colors® pAcGFP1-C1 Vector[#]	Clontech/Takara	632470
Corning® CellBIND® 6-well Clear Multiple Well Plates	Corning	3335
Corning® CellBIND® 24-well Clear Multiple Well Plates	Corning	3337
Corning® CoolCell® Freezing System	Corning	UX-04392-00

The above reagents are recommended based on our culture protocols. If you are using a similar/ alternate reagent, we recommend that you perform a small-batch test using your preferred reagents. [#] Any GFP plasmid with size <7kb can be used.

Protocol

1. Preparation of culture medium

1.1 Example preparation of HEK293 medium (complete growth medium)

- 1000 mL DMEM High Glucose, (-) Glutamine, (-) Pyruvate
- 50 ml FBS
- 11 mL 100x GlutaMAX
- 11 mL 100 mM Pyruvate
- 27.4 mL 1 M HEPES

Notes:

- *The medium should be kept at 4°C. Only aliquots that will be used up that day are warmed up to room temperature or 37°C.*
- *Pen/Strep (Gibco™ 15140) is diluted 200x into the aliquot that will be used. It is *not* added to the stock medium.*
- *Pen/Strep aliquots must be stored at -20°C in a non-cycling freezer (i.e., freezers that build-up ice). Aliquots are only thawed once.*

1.2 Freezing medium

- 90% HEK293 medium
- 10% DMSO

1.3 Example preparation of sorting medium: (for FACS recovery)

- 10.00 mL HEK293 medium
- 0.53 mL FBS
- 53 µL Pen/Strep
- 22 µL Normocin

2. Thawing and culturing cryopreserved cells

To ensure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

2.1 Thaw the vial containing HEK293 cells by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2.2 Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.
Note: All of the operations from this point on should be carried out under strict aseptic conditions.
2.3 Transfer the contents of the vial equally into 2 wells of a 6-well plate (1/2 vial in each well; 2-fold dilution).
2.4 Add “enriched” complete growth medium (complete growth medium in step 1.1 but with 9% FBS) while moving the plate in a figure-8 pattern several times.
2.5 Incubate the culture in a suitable incubator at 37°C in 5% CO_2 for 24 hours.
2.6 After 24 hours, change media to complete growth medium (step 1.1).
2.7 Incubate the culture at 37°C in 5% CO_2.

3. Sub-culturing procedure (maintenance)

Volumes are given for a 10 cm^2 well in a multi-well plate (6-well plate). Corning® CellBIND® 6-well Clear Multiple Well Plates or poly-lysine coated plates are highly recommended for subculturing this product. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

3.1 Remove and discard the spent culture medium.

3.2 Add 750 µL of TrypLE solution to one well and observe cells under an inverted microscope until the cell layer is dispersed (usually 3-5 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the plate while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
3.3 Add 750 µL of complete growth medium and swirl to mix. Then break-up the cell clumps by gently pipetting.
3.4 Add appropriate aliquots of the cell suspension to new culture vessels.
3.5 Incubate cultures in a 37°C/ 5% CO_2 incubator.
3.6 Subculture before cell confluency reaches 100%.
- Subcultivation ratio: 1:6 to 1:20 weekly, depending on timing of expected usage
- Medium renewal: Every 2 to 3 days

4. Cryopreserving cells

We recommend cryopreserving the cells from a 70-90% confluent well of a 6-well plate (i.e., 10 cm^2 growth area) in 1 mL of freezing medium per cryogenic vial.

4.1 Remove the medium (supernatant) from culture well or flask.
4.2 Add adequate amount of freezing media to cells (1 mL for 10 cm^2 growth area), and detach the cells by pipetting.
4.3 Transfer approximately 1 to 1.5 mL of the cell suspension to a labeled cryogenic vial.
4.4 Move the vial(s) to a Corning® CoolCell® Freezing System for cryogenic vials and move the CoolCell® holder to a -80°C freezer.
Note: Try to place the vials symmetrically in CoolCell® holder to facilitate even freezing across all vials.
4.5 After one day, move the cryogenic vials to liquid nitrogen storage.
Note: Place the cryogenic vials on dry-ice while transferring from -80°C freezer to the liquid nitrogen tank.

5. Transfection procedure

5.1 Cloning with TARGATT™ 54 attB-mCherry-LZ Cloning Plasmid

5.1.1 Clone the insert or insert-variant library of interest into the TARGATT™ pKHESL-mCherry-LZ (library) Cloning Plasmid to make the donor plasmid or pool using the NEB® Golden Gate Assembly Kit (BsaI-HF®v2).
Note: See appendix for an example of how to design your cloning/library primers.
5.1.2 Transform the plasmid(s) into E. cloni® 10G SUPREME SOLOs electrocompetent cells or a comparable DH10B line.
Note 1: See the E. cloni® 10G Electrocompetent Cells manual for the transformation protocol.
Note 2: Do not use 5-alpha competent cells to transform the plasmids, they are sub-optimal for vectors this large.
5.1.3 Purify plasmid(s) and store at -20°C. Minimize repeated freeze-thaw cycles (i.e. make aliquots if this is needed).

5.2 Seeding for transfection the next day in a 24-well plate

5.2.1 Warm up the following reagents at room temperature:
- Aliquot of HEK293 medium without Pen/Strep
- Pen/Strep
- TrypLE
- DPBS (for resuspending cells after centrifugation)

5.2.2 Aspirate the medium (supernatant) from the culture vessel.
5.2.3 Immediately add 750 µL TrypLE per well of a 6-well plate.
5.2.4 Allow the trypsinization to work for ~2-5 minutes.
5.2.5 Add 750 µL complete growth medium and swirl to mix. Then break-up the cell clumps by pipetting.
5.2.6 Transfer the entire content of the well to a labeled 1.5 mL centrifuge tube.
5.2.7 Centrifuge at 200 x g for 5 minutes.

5.2.8 Aspirate most of the supernatant, leaving a little liquid at bottom. Use a pipette to remove the remaining supernatant without disturbing cell pellet.

5.2.9 Resuspend in 1 mL DPBS and mix by pipetting a couple of times.
Note: At this point, if necessary, combine resuspended cells from multiple wells in a 15 mL tube for counting.

5.2.10 Count cells using trypan blue and an automated cell counter.

5.2.11 Dilute the cells in no-antibiotic growth medium such that their concentration will be 0.4 x 10^6 cells/mL.

5.2.12 Pipet 0.5 mL of the cell solution into each desired well of the 24-well plate (i.e., 200,000 live cells per 2-cm^2 well). If this is the first time that the cells are being seeded, test additional densities (e.g. 175k and 225k) to compensate for potential differences between cell counters, humidity levels, etc.

5.2.13 Distribute the cells evenly by quickly moving the plate in a figure-8 pattern several times.

5.2.14 Incubate at 37°C in 5% CO_2 overnight.

5.2.15 The next day, only proceed with transfection in wells where the confluency is in the 60-80% range.

5.3 Transfection using Xfect™ Transfection Reagent

5.3.1 Warm up the following reagents at room temperature:
- Xfect™ Transfection Reagent (stored at 4°C)
- Xfect™ Transfection Buffer (stored at 4°C)

5.3.2 Calculate the volume of the Xfect™ transfection reagent, buffer and plasmid volume to be mixed.
Note: The Xfect™ to plasmid ratio is 0.3 µL Xfect™ per 1 µg plasmid. However, do not pipet less than 1µL (i.e., always prepare at least a 3.33 µg plasmid mix, even if only transfecting 2 µg, 1 µg, etc.).

Table 1. Example transfection mix for transfecting 2 µg GFP plasmid into three wells of a 24-well plate for reagent validation[#]

Reagent	Amount
GFP Plasmid*	6.6 µg**
Xfect™ transfection reagent	1.98 µL
Xfect™ buffer	q.s.
Final volume	**109.9 µL**

** Any GFP plasmid < 7kb can be used. ** Amount of plasmid DNA to be used is given in µg quantity. Calculate the appropriate volume based on the concentration of the plasmid. [#] Amount of transfection mix recommended for pipetting 33.3 µL in three wells.*

5.3.3 For integrase-mediated integration transfections, we recommend a 3:1 donor:integrase mass ratio (i.e., three-fold more of the donor by mass).

Table 2. Transfection of donor or positive control plasmid into three wells of a 24-well plate[#]

Reagent	Amount
Integrase plasmid	1.65 µg**
Donor or positive control plasmid	4.95 µg**
Xfect™ transfection reagent	1.98 µL
Xfect™ buffer	q.s.
Final volume	**109.9 µL**

*** Amount of plasmid DNA to be used is given in µg quantity. Calculate the appropriate volume based on the concentration of the plasmid. [#] Amount of transfection mix recommended for pipetting 33.3 µL in three wells.*
Note: Quantum satis (q.s.), the volume needed to bring the final solution volume to 109.9 µL

5.3.4 Label 1.5 mL microcentrifuge tubes.
5.3.5 If the medium that the cells are in has Pen/Strep, carefully replace it with warm non-Pen/Strep medium.
5.3.6 Add calculated amount of Xfect™ buffer and plasmid to the microcentrifuge tube.
5.3.7 Vortex or pipet the Xfect™ reagent to fully resuspend it, and then transfer the calculated amount of Xfect™ into the plasmid solution.
5.3.8 Quickly vortex the transfection solution to mix it well. Allow it to incubate at room temperature for at least 10 minutes (but no longer than 15 minutes).
5.3.9 Transfer the appropriate amount of mix to each well of the culture plate without disturbing the attached cells. (For example, to transfect 2 µg plasmid DNA, pipet 33.3 µL of the transfection mix into each desired well of the 24-well plate).
Note: Give a quick shake immediately after transferring the transfection mix to each well.
5.3.10 Incubate at 37°C/ 5% CO_2 for 72 hours. We have observed the highest integration efficiencies 72-96 hours after transfection (no medium change is required for 72 hours).
5.3.11 After the 72-hour incubation, cells are ready to be sorted.
Note: When sorting, use sorting medium (step 1.3) for robust recovery of cells.

Supporting Data

mCherry2 expression 96 hours post-transfection

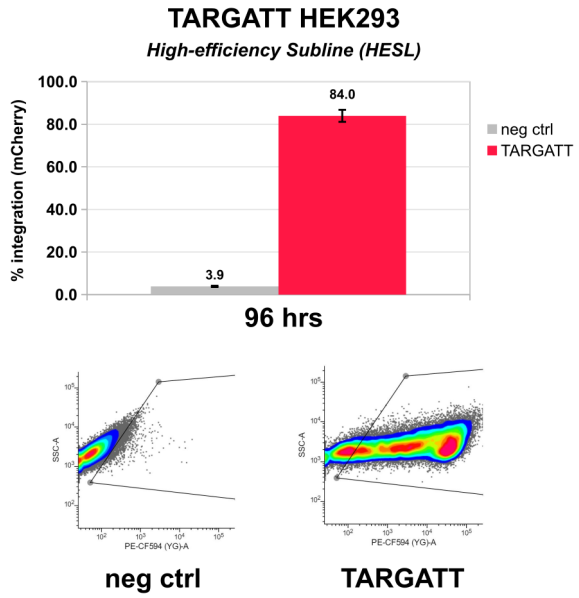

Figure 2. pT1-mCh2-HESL Positive Control plasmid integration into the HESL TARGATT™ HEK293 master cell line mediated by integrase, 96 hours post-transfection. Cells were co-transfected with the mCherry2 positive control plasmid and either the provided TARGATT™ integrase plasmid (TARGATT) or a no-integrase control plasmid (neg ctrl). The pT1-mCh2-HESL plasmid has no promoter and requires the EF1 promoter in the landing pad after integration to express mCherry2. The integration efficiency of the 4.2 kb plasmid into the landing pad was 80%, without selection. Data represents the mean ± SE of a representative high-transfection-efficiency experiment (98% as judged by CMV-GFP plasmid) done in triplicate.

Appendix

Primer Design Example

These primers are for BsaI Golden Gate cloning which require that your library not have a BsaI site. If necessary, alternative primers can be designed using other IIS restriction enzymes. If you choose to use other restriction enzymes, please contact Applied StemCell for assistance.

Primer	Sequence
Forward Primer	5' gtGGTCTCtTCCTatg ... 3'
*Where 'atg' is the start codon of your protein, and '...' is the rest of the primer that is specific to your coding sequence.	
Reverse Primer	5' caGGTCTCaTAAGcta ... 3'
*Where 'cta' is the reverse complement of the 'tag' stop codon, and '...' is the rest of the primer that is specific to the reverse complement of your coding sequence.	