Applied StemCell, Inc.
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Datasheet

HEK293 mCherry Cell Line

Product Information

Catalog Number	**AST-1320**
Description	Applied StemCell, Inc. provides high-quality mCherry expressing HEK293 cells. The HEK293 mCherry cells were generated via the integration of mCherry into the TARGATT™ HEK293 Master Cell Line with the use of a unique integrase and an mCherry control plasmid.
Parental Cell Line	HEK293 Adherent Cells
Quantity	1 x 10^6 cells/vial
Shipping	Dry ice
Storage and Stability	Store the HEK293 mCherry Cell Line in a liquid nitrogen freezer immediately upon receipt. The cells provided are stable for at least 1 year from the date of receiving when stored as directed.
Quality Control	A certificate of analysis (COA) with detailed quality control information for the cell line and components of the kit will be provided with each shipment
Safety Precaution	**PLEASE READ BEFORE HANDLING ANY FROZEN VIALS**. Please wear appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling frozen vials. 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.

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Characterization of AST-1320

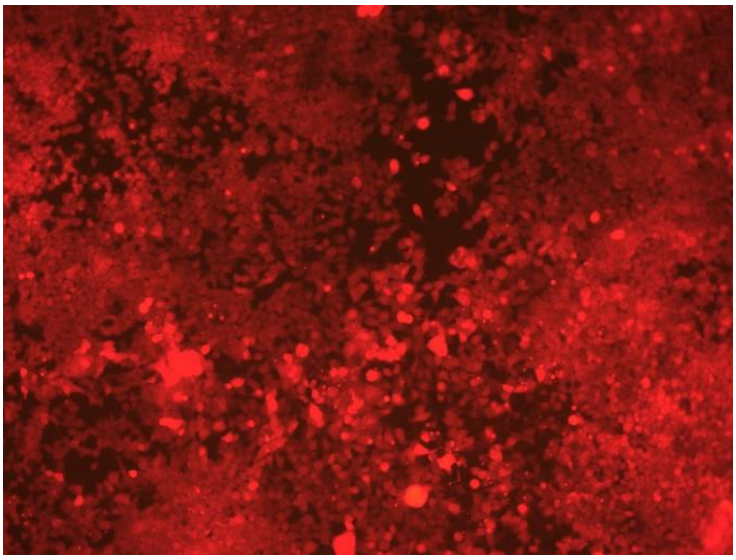

Figure 1. The AST-1320 HEK293 Cell Line Expresses mCherry. mCherry signal was detected by fluorescence imaging. Image taken at 20x magnification.

Materials

Catalog#	Component	Amount
AST-1320	HEK293 mCherry Cell Line	1 x 10^6 cells

Media and Material Required 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
Gibco™ Penicillin-Streptomycin (10,000 U/mL)	ThermoFisher	15140148
Normocin™ - Antimicrobial Reagent, 500 mg	Invivogen	ant-nr-1
DPBS, No Calcium, No Magnesium	ThermoFisher	14190136
0.25% Trypsin EDTA (1x)	ThermoFisher	25200056
Dimethyl Sulfoxide (DMSO)	Sigma-Aldrich	D2438
Corning® CellBIND® 6-well Clear Multiple Well Plates	Corning	3335
Corning® CoolCell® Freezing System	Corning	UX-04392-00

Protocol

1. Preparation of culture medium

1. **Example preparation of HEK293 medium (complete growth medium)**
   a. 930 mL DMEM High Glucose, (-) Glutamine, (-) Pyruvate
   b. 50 ml FBS
   c. 10 mL 100x GlutaMAX
   d. 10 mL 100mM Pyruvate

Notes:

*e. 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.*
*f. Pen/Strep (Gibco™ 15140) is diluted 200x into the aliquot that will be used. It is *not* added to the stock medium.*
*g. 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.*

2. **Freezing medium**
   a. 90% HEK293 medium
   b. 10% DMSO

3. **Example preparation of sorting medium: (for FACS recovery)**
   a. 10.00 mL HEK293 medium
   b. 0.53 mL FBS
   c. 53 µL Pen/Strep
   d. 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 4-fold diluted 0.25% Trypsin-EDTA (T/4) solution (diluted with DPBS) 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 the 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.