

## HEK293/Human ASGR1&ASGR2 Stable Cell Line

Catalog No.	Size	
CHEK-ATP172	$2 \times (1 \text{ vial contains } \sim 5 \times 10^6 \text{ cells})$	

### • Description

The HEK293/Human ASGR1&ASGR2 Stable Cell Line was engineered to express the receptors full length human ASGR1 (Gene ID: 432) and ASGR2 (Gene ID: 433). Surface expression of human ASGR1 and ASGR2 was confirmed by flow cytometry.

### • Application

Useful for cell-based ASGR1 or ASGR2 binding assay.

### • Cell Line Profile

Cell line	HEK293/Human ASGR1&ASGR2 Stable Cell Line		
Host Cell	HEK293		
Property	Adherent		
Complete Growth Medium	DMEM + 10% FBS		
Selection Marker	Puromycin (2 μg/mL) + Hygromycin (10 μg/mL)		
Incubation	37°C with 5% CO <sub>2</sub>		
Doubling Time	22-24 hours		
Transduction Technique	Lentivirus		



### • Materials Required for Cell Culture

- DMEM medium (Gibco, Cat.No.11965-092)
- Fetal bovine serum (CellMax, Cat.No.SA211.02)
- Puromycin (InvivoGen, Cat.No.ant-pr-5b)
- Hygromycin B (InvivoGen, Cat.No.ant-hg-1)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 μg/mL), Hygromycin (10 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-II)
- CO<sub>2</sub> Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)



### • Recovery

- 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 2 minutes).
- 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
- 3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium and spin at approximately 1000 rpm for 5 minutes.
- 4. Resuspend cell pellet with 5 mL complete growth medium and transfer the cell suspension into T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
- 5. Incubate at 37°C with 5% CO<sub>2</sub> incubator until the cells are ready to be split.

#### • Subculture

- 1. Remove and discard culture medium.
- 2. Wash the cells once with sterile PBS.
- 3. Add 2 mL of 0.25% trypsin to cell culture flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached.
- 4. Add 6.0 to 8.0 mL of culture medium and aspirate cells by gently pipetting.
- 5. Add appropriate aliquots of the cell suspension to new culture vessel.
- 6. Incubate at 37°C with 5% CO<sub>2</sub> incubator.

**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:5 is recommended.

**Medium Renewal:** Every 2 to 3 days.



### • Cryopreservation

- 1. Remove and discard spent medium.
- 2. Detach cells from the cell culture flasks with 0.25% trypsin.
- 3. Centrifuge at 1000 rpm for 5 min at RT to pellet cells.
- 4. Resuspend the cell pellets with complete growth medium and count viable cells.
- 5. Centrifuge at 1000 rpm for 5 min at RT and resuspend cells in freezing medium to a concentration of  $5\times10^6$  to  $1\times10^7$  cells/mL.
- 6. Aliquot into cryogenic storage vials. Place vials in a programmable cooler or an insulated box placed in a  $-80^{\circ}$ C freezer overnight, then transferring to liquid nitrogen storage.

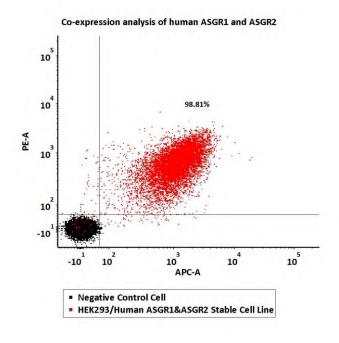
### • Storage

Product format: Frozen

• Storage conditions: Liquid nitrogen immediately upon receipt



### • Receptor Assay



Catalog No.	Stable Cell Line	(ASGR1 <sup>+</sup> ASGR2 <sup>+</sup> ) %	MFI for ASGR1 (APC)	MFI for ASGR2 (PE)
NA	Negative Control Cell	0.01	1	1
CHEK-ATP172	HEK293/Human ASGR1&ASGR2 Stable Cell Line	98.81	1104	633

Fig1. Co-expression analysis of human ASGR1 and ASGR2 on HEK293/Human ASGR1&ASGR2 Stable Cell Line by FACS. Cell surface staining was performed on HEK293/Human ASGR1&ASGR2 Stable Cell Line or negative control cell using APC-labeled anti-ASGR1 antibody and PE-labeled anti-ASGR2 antibody.

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<u>Products</u> <u>Cat.No.</u>

HEK293/Human ASGR1 Stable Cell Line

CHEK-ATP080