

### Human c-MET (Luc) HEK293 Reporter Cell

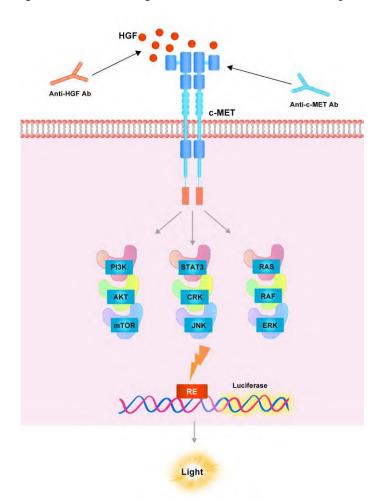
Catalog No.	Size
CHEK-ATF144	$2 \times (1 \text{ vial contains } \sim 5 \times 10^6 \text{ cells})$

#### • Description

The Human c-MET (Luc) HEK293 Reporter Cell was engineered to express signaling response element driving luciferase expressing systems and human c-MET (Gene ID: 4233). When stimulated with human HGF protein, the HGF/c-MET interaction drives RE-mediated luminescence. Neutralization of biological effect of human HGF protein by corresponding antibody results in a decrease in luminescence.

### • Application

• Screen for neutralizing antibodies blocking the stimulation of human HGF protein.





### • Cell Line Profile

Cell line	Human c-MET(Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Puromycin (2 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)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Puromycin (2 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 1:6 to 1:10 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°C freezer overnight, then transferring to liquid nitrogen storage.

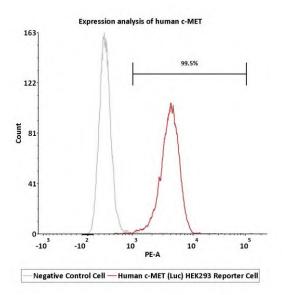
#### • Storage

Product format: Frozen

• Storage conditions: Liquid nitrogen immediately upon receipt



#### • Receptor Assay



**Fig1.** Expression analysis of human c-MET on Human c-MET (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human c-MET (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-human c-MET antibody.

#### • Signaling Bioassay

### **Human HGF Protein Stimulation (RLU)**

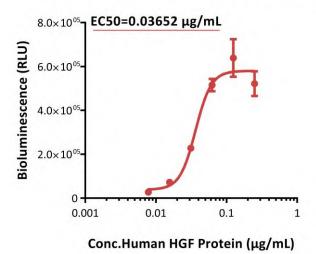
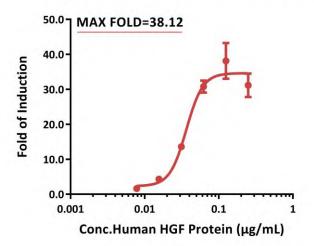


Fig2. Response to human HGF protein (RLU). This reporter cell was incubated with serial dilutions of human HGF protein. The EC<sub>50</sub> was approximately  $0.03652 \, \mu \text{g/mL}$ .



### **Human HGF Protein Stimulation (FOLD)**



**Fig3. Response to human HGF protein (FOLD).** This reporter cell was incubated with serial dilutions of human HGF protein. The max induction fold was approximately 38.12.

### Application

#### **Anti-human c-MET Neutralizing Antibody Screening**

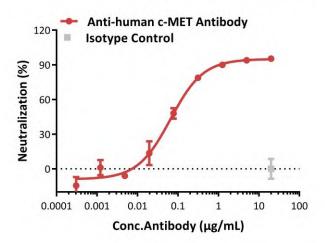


Fig4. Inhibition of human HGF protein-induced reporter activity by anti-human c-MET neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human HGF protein with a final concentration of  $0.05 \mu g/mL$ . The EC50 of anti-human c-MET neutralizing antibody (Amivantamab) is approximately  $0.06561 \mu g/mL$ .



### • License Disclosure

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#### • Related Products

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

Human EGF R (Luc) HEK293 Reporter Cell CHEK-ATF049