NFAT (Luc) HEK293 Reporter Cell Data Sheet

NFAT (Luc) HEK293 Reporter Cell

Catalog No.	Size
CHEK-ATF050	1 vial contains ~5×10^6 cells

• *Description*

The NFAT (Luc) HEK293 Reporter Cell was engineered with the NFAT response element driving luciferase expressing systems. The receptors expressing endogenously or transfected on this reporter cell were activated by corresponding ligands binding, transducing intracellular signals resulting in NFAT-RE mediated luminescence.

• *Cell Line Profile*

Cell line	NFAT (Luc) HEK293 Reporter Cell
Species	Human
Property	Adherent
Medium	DMEM + 10% FBS
Selection Marker	Hygromycin (50 μg/mL)
Incubation	37°C with 5% CO_2
Storage	Frozen in liquid nitrogen
Doubling Time	22-24 hours
Biosafety Level	1
Application	1. The discovery of activators or inhibitors by the NFAT signaling bioactivity 2. Transfection host for some receptors concerning the NFAT signaling pathway

• *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℃ with 5% CO_2 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 complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessel.
6. Incubate at 37℃ with 5% CO_2 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 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 5×10^6 to 1×10^7 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.

Freezing medium:

- 10~90% FBS
- 10% DMSO
- 0~70% DMEM medium

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- ***Signaling Bioassay***

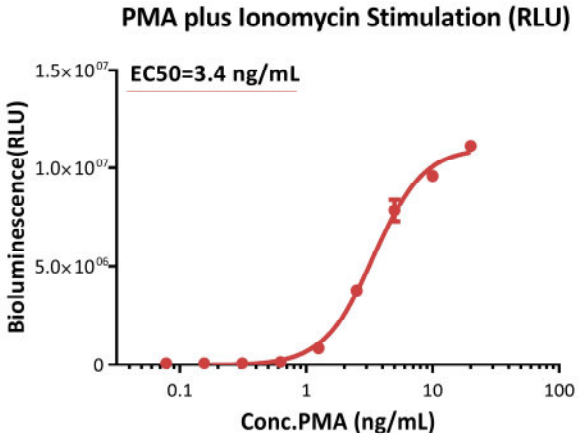

Fig1. Response to PMA plus Ionomycin (RLU). The NFAT (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of PMA plus Ionomycin (2 μM). The EC50 was approximately 3.4 ng/mL.

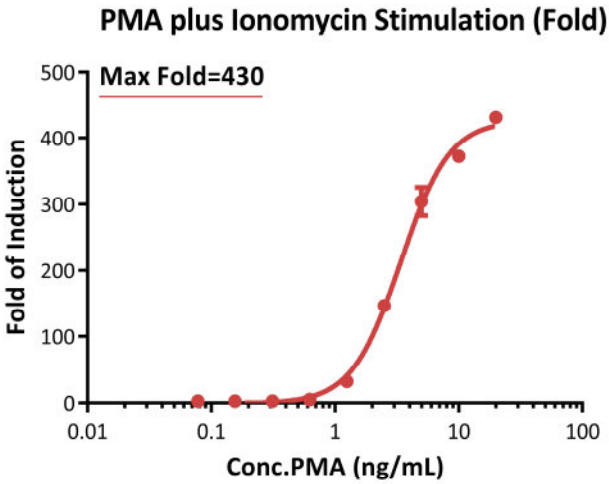

Fig2. Response to PMA plus Ionomycin (Fold). The NFAT (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of PMA plus Ionomycin (2 μM). The max induction fold was approximately 430.

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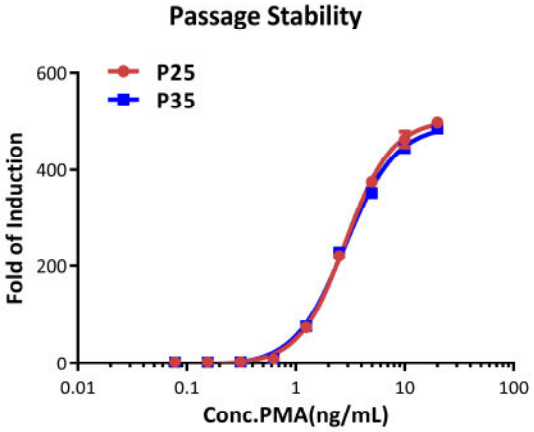
- ***Passage Stability***



	P25	P35
EC50 (ng/mL)	2.872	2.852
Max Fold	498	485

Fig3. Passage stability analysis by Signaling Bioassay. The continuously growing NFAT (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of PMA plus Ionomycin (2 μM). PMA plus Ionomycin stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 25-35. And, the bioactivity of NFAT signaling still can be detected by PMA plus Ionomycin stimulation on passage 35 with high activating fold.