Human VEGF R2 (Luc) HEK293 Reporter Cell Data Sheet

Human VEGF R2 (Luc) HEK293 Reporter Cell

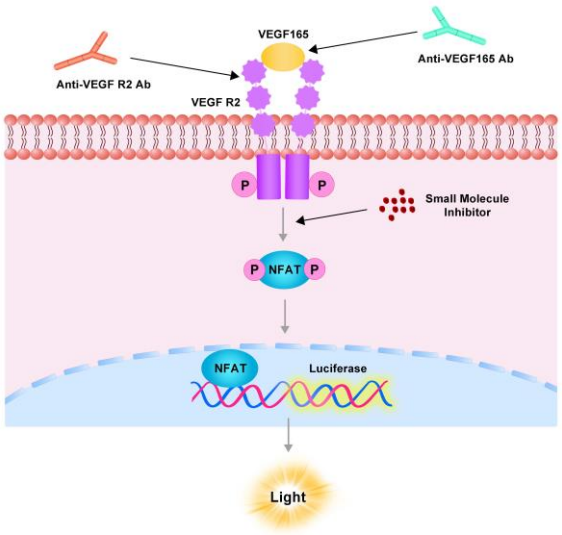
Catalog No.	Size
CHEK-ATF044	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human VEGF R2 (Luc) HEK293 Reporter Cell was engineered to not only express NFAT signaling response element, but also express the receptor full length human VEGF R2 (Gene ID: 3791). When stimulated with human VEGF protein, the VEGF/VEGF R2 interaction drives NFAT-mediated luminescence. Inhibition of VEGF binding to VEGF R2 by either anti-VEGF or anti-VEGF R2 antibodies results in a decrease in luminescence.

• *Application*

- Screen for anti-human VEGF R2 or anti-human VEGF neutralizing antibody.
- Screen for human VEGF R2 small molecule inhibitor



• *Cell Line Profile*

Cell line	Human VEGF R2 (Luc) HEK293 Reporter Cell
Host Cell	HEK293
Property	Adherent
Complete Growth Medium	DMEM + 10% FBS
Selection Marker	Hygromycin (50 μg/mL) + Puromycin (5 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

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• *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 (Invitrogen, Cat.No.10687010)
- Complete Growth Medium: DMEM + 10% FBS
- Culture Medium: DMEM + 10% FBS, Hygromycin (50 μg/mL), Puromycin (5 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_2 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_2 incubator until the cells are ready to be split.

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• *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℃ 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 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 5×10^6 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.

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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- ***Receptor Assay***

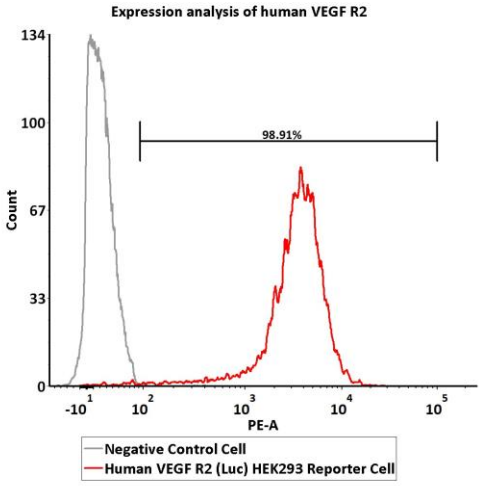

Fig1. Expression analysis of human VEGF R2 on Human VEGF R2 (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human VEGF R2 (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-VEGF R2 antibody.

- ***Signaling Bioassay***

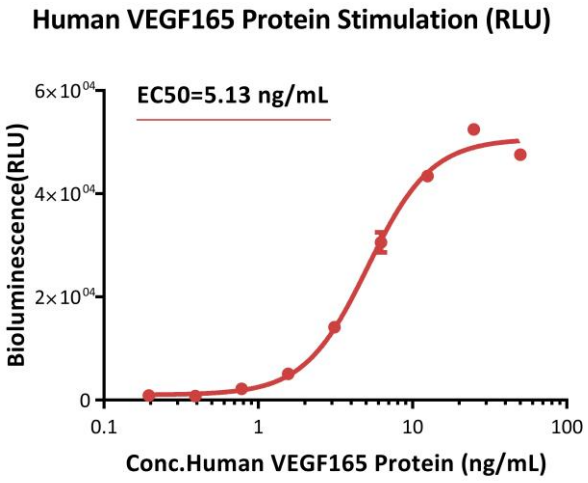

Fig2. Response to human VEGF165 protein (RLU). The Human VEGF R2 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human VEGF165 protein（Cat.NO.VE5-H4210）. The EC50 was approximately 5.13 ng/mL.

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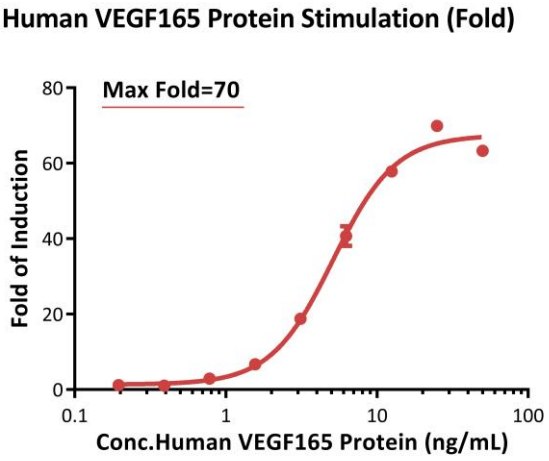

Fig3. Response to human VEGF165 protein (Fold). The Human VEGF R2 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human VEGF165 protein（Cat.NO.VE5-H4210）. The max induction fold was approximately 70.

- ***Application***

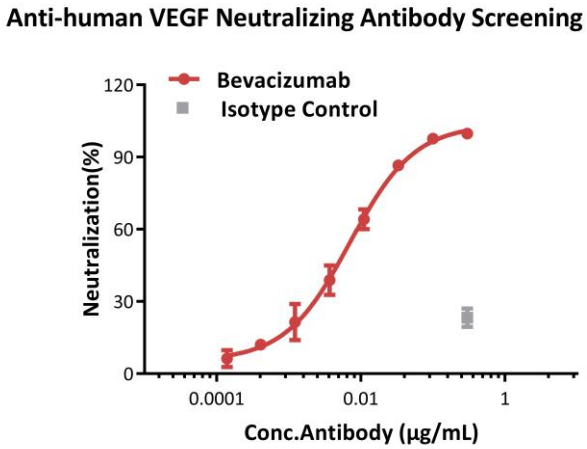

Fig4. Inhibition of human VEGF165 protein-induced reporter activity by anti-human VEGF neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human VEGF165 protein（Cat.NO.VE5-H4210）with a final concentration of 10 ng/mL. The EC50 of anti-human VEGF neutralizing antibody (Bavacizumab) is approximately 0.0071 μg/mL.

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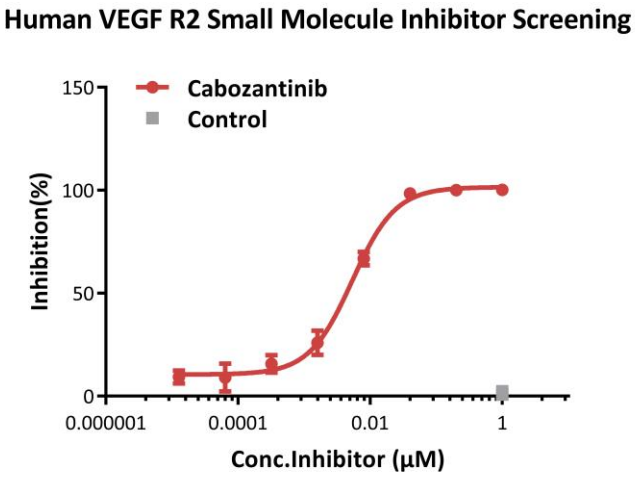
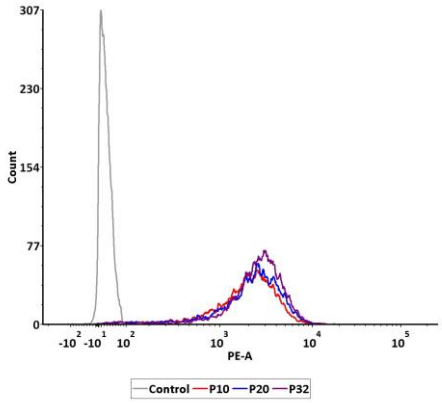


Fig5. Inhibition of human VEGF165 protein-induced reporter activity by human VEGF R2 small molecule inhibitor. This reporter cell was incubated with serial dilutions of inhibitors in the presence of human VEGF165 protein (Cat.NO.VE5-H4210) with a final concentration of 10 ng/mL. The EC50 of human VEGF R2 small molecule inhibitor (Cabozantinib) was approximately 0.0053 μM.

- ***Passage Stability***



Passage	MFI for VEGF R2 (PE)
P10	1993.09
P20	2317.72
P32	2566.44

Fig6. Passage stability analysis of receptor expression by FACS. Flow cytometry surface staining of human VEGF R2 on Human VEGF R2 (Luc) HEK293 Reporter Cell demonstrates consistent mean fluorescent intensity across passage10-32.

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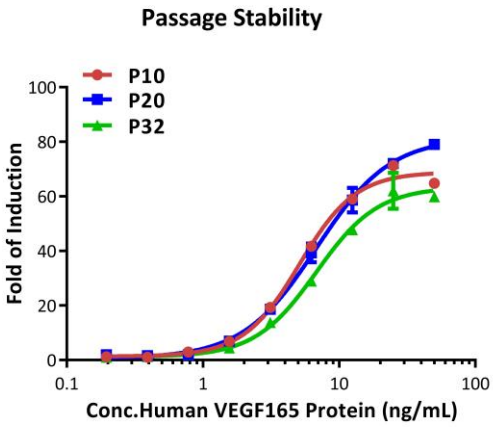

	P10	P20	P32
EC50 (ng/mL)	5.115	6.813	6.805
Max Fold	71	79	62

Fig7. Passage stability analysis by Signaling Bioassay. The continuously growing Human VEGF R2 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human VEGF165 protein. Human VEGF165 protein stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 10-32.

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• *Related Products*

Products	**Cat.No.**
Human VEGF165 protein	VE5-H4210