Human VEGF R2 (Luc) HEK293 Reporter Cell Data Sheet

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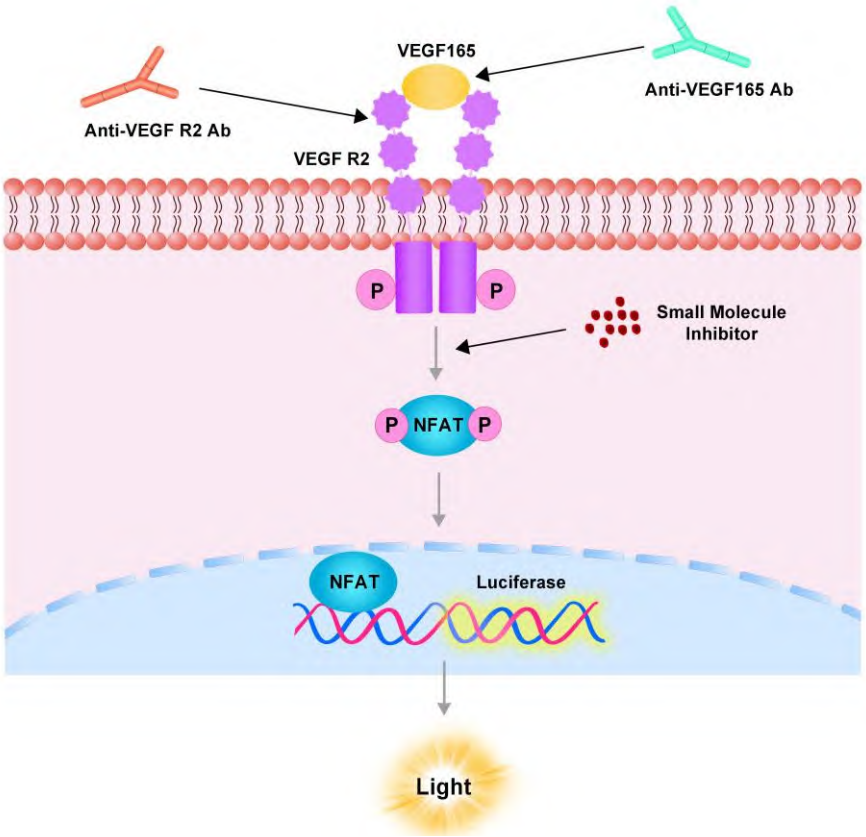
Catalog No.	Size
CHEK-ATF044	2 × (1 vial contains ~5×10^6 cells)

• *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 (Uniprot: P35968-1). 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.



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• *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 B (20 μg/mL) + Puromycin (2 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- DMEM Medium (BasalMedia, Cat. No. L120KJ)

Note: If you are unable to obtain the specified DMEM medium (BasalMedia, Cat. No. L120KJ) in China, you may use an alternative DMEM medium (Gibco, Cat. No. 11965-092) or another suitable medium for culturing.

- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: DMEM + 10% FBS, + 1%PS
- Culture Medium: DMEM + 10% FBS, Hygromycin B (20 μg/mL), Puromycin (2 μg/mL), + 1%PS
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO_2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

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• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a 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.

• *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:4 to 1:8 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% CO_2 incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition, transition to the culture medium containing the selection marker during subculturing.

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• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 2 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 2-3 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage Condition*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

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- ***Receptor Assay***

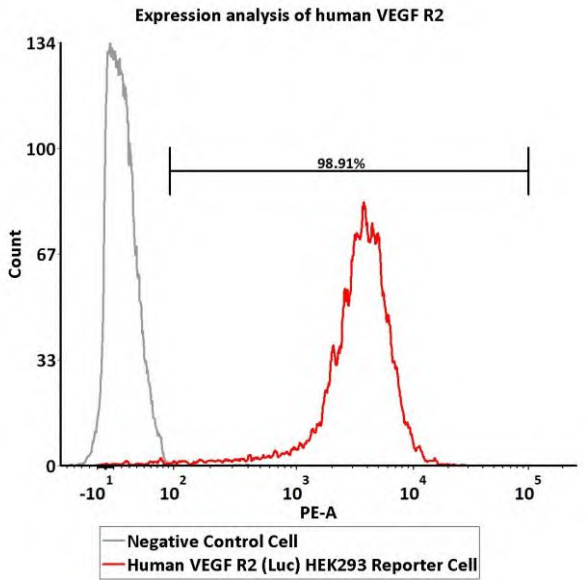

Fig1. Expression analysis of human VEGF R2 on Human VEGF R2 (Luc) HEK293 Reporter Cell by FACS. Cell surface staining was performed on Human VEGF R2 (Luc) HEK293 Reporter Cell or negative control cell using PE-labeled anti-VEGF R2 antibody.

- ***Signaling Bioassay***

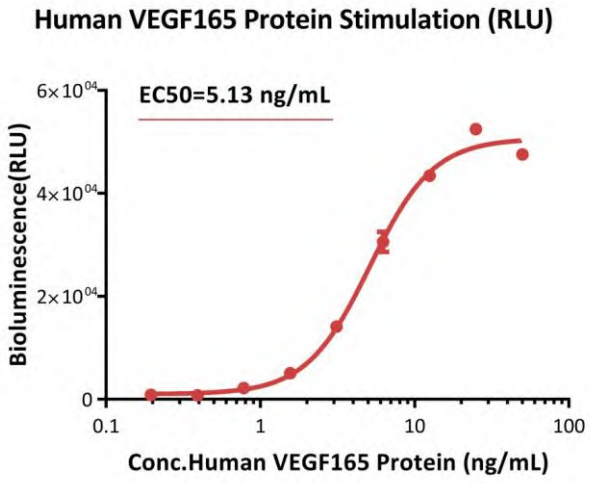

Fig2. Response to human VEGF165 protein (RLU). The Human VEGF R2 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human VEGF165 protein (Cat. No. VE5-H4210). The EC50 was approximately 5.13 ng/mL.

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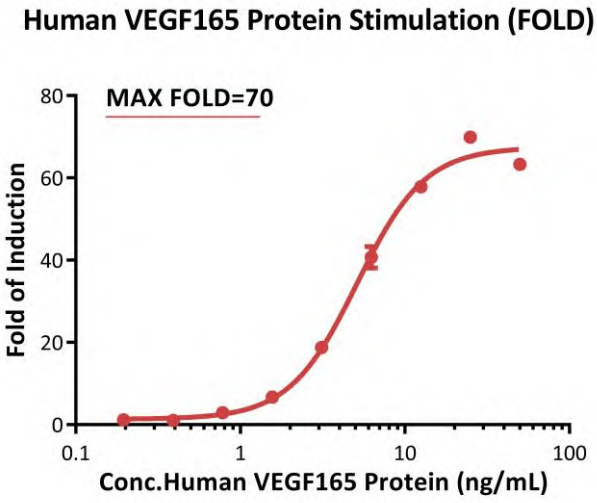

Fig3. Response to human VEGF165 protein (FOLD). The Human VEGF R2 (Luc) HEK293 Reporter Cell was stimulated with serial dilutions of human VEGF165 protein（Cat. No. VE5-H4210）. The max induction fold was approximately 70.

- ***Application***

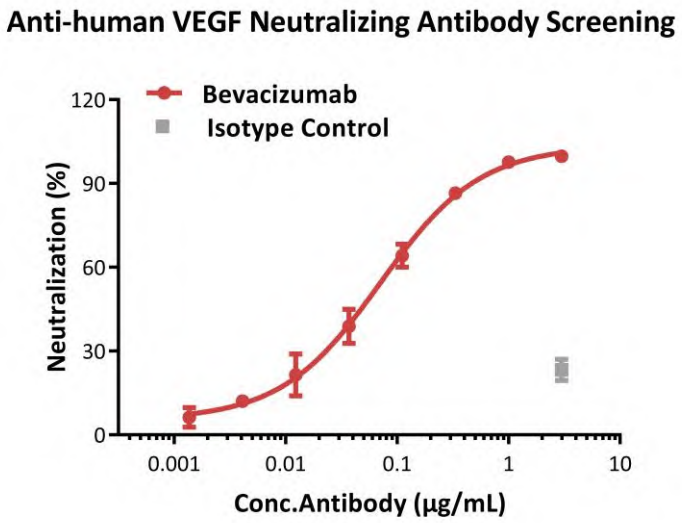

Fig4. Inhibition of human VEGF165 protein-induced reporter activity by anti-human VEGF neutralizing antibody. This reporter cell was incubated with serial dilutions of antibodies in the presence of human VEGF165 protein（Cat. No. VE5-H4210） with a final concentration of 10 ng/mL. The EC50 of anti-human VEGF neutralizing antibody (Bevacizumab) is approximately 0.071 μg/mL.

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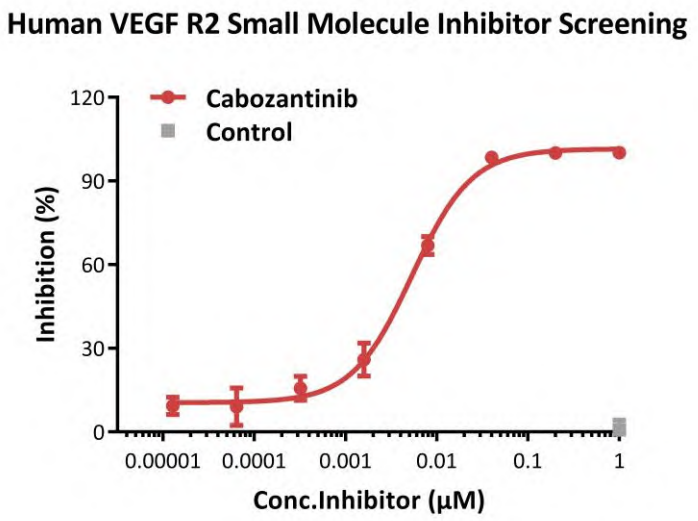

Fig5. Inhibition of human VEGF165 protein-induced reporter activity by human VEGF R2 small molecule inhibitor. This reporter cell was incubated with serial dilutions of inhibitors in the presence of human VEGF165 protein (Cat. No. VE5-H4210) with a final concentration of 10 ng/mL. The EC50 of human VEGF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

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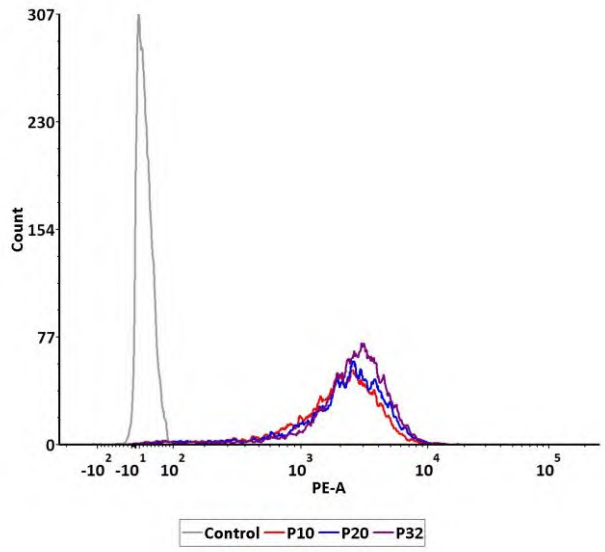
- ***Passage Stability***



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 passage 10-32.

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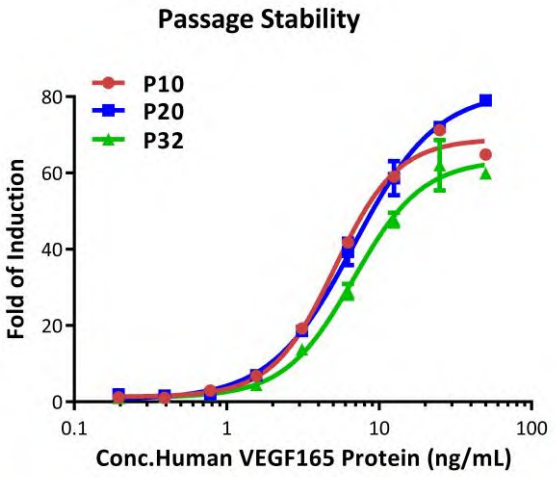

	P10	P20	P32
EC50 (ng/mL)	5.115	6.813	6.805
Max Fold	71	79	62

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 (Cat. No. VE5-H4210). 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
NF- κ B (Luc) HEK293 Reporter Cell	CHEK-ATF048
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
HEK293/Human CCR5 Stable Cell Line	CHEK-ATP043
HEK293/Human SIRP alpha Stable Cell Line	CHEK-ATP051
HEK293/Human CD20 Stable Cell Line	CHEK-ATP034
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human TMPRSS2-HA-P2A-mGFP Stable Cell Line	CHEK-ATP101
NF-kB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
NY-ESO-1 specific TCR-HEK293 cell line	CHEK-STP114
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
HEK293/Human CCR8 Stable Cell Line	CHEK-ATP140
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
HEK293/Human ASGR1&ASGR2 Stable Cell Line	CHEK-ATP172
Human BMP (Luc) HEK293 Reporter Cell	CHEK-ATF188
HEK293/Human IDH1(132H)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP199
HEK293/Human IDH1(132R)-P2A-mGFP&Luc Stable Cell Line	CHEK-ATP200