Description

VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line is a HEK293 cell line expressing the firefly luciferase reporter under the control of NFAT response elements. The cells also stably express human VEGFR2 (Human Vascular Endothelial Growth Factor Receptor 2; KDR, FLK1; ref seq. NM_002253.1).

Expression of VEGFR2 was assessed by flow cytometry and this cell line has been functionally validated with Sorafenib, Anti-VEGF Neutralizing Antibody and Aflibercept Biosimilar.

Background

The interaction between VEGF (Vascular Endothelial Growth Factor) and its receptor VEGFR is an important mediator of angiogenesis as well as endothelial cell survival and proliferation. VEGFR2 is a receptor tyrosine kinase that plays a major role in cellular responses driven by VEGF. Targeting the kinase activity of VEGFR2 has been an attractive tool in cancer therapy based on its anti-angiogenesis effects. In addition to the kinase inhibitors, humanized monoclonal antibodies interfering with VEGF/VEGFR2 interaction have been also developed for anti-cancer treatments.

Application(s)

- Screen for activators or inhibitors of VEGF/VEGFR2 signaling pathway
- Screen for inhibitors of VEGFR2 kinase activity

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1A	BPS Bioscience #79528

Materials Used in the Cellular Assays

Name	Ordering Information
Human VEGF165	BPS Bioscience #91001
Thaw Medium 1	BPS Bioscience #60187
Protease-free Bovine Serum Albumin (BSA)	Sigma-Aldrich #A4919
Sorafenib	LC Laboratories #S-8599
Anti-VEGF Neutralizing Antibody	BPS Bioscience #79478
Aflibercept Biosimilar	Ichorbio #ICH4015
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture treated white clear-bottom assay plate	Corning #3610
Luminometer	

Storage Conditions

Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage.
Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1A (BPS Bioscience #79528):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 400 µg/ml of Geneticin, and 100 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Assay Medium:
Prepare the Assay Medium using DMEM medium supplemented with 1% (w/vol) protease free BSA (Sigma-Aldrich #A4919).

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 1.

   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1A.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1A.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1A and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

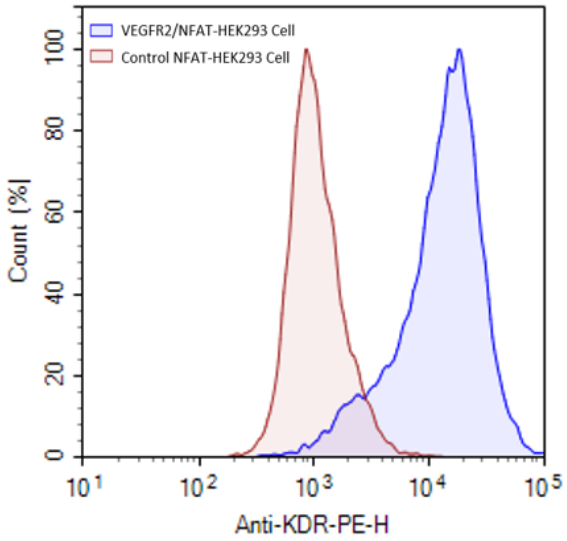

Figure 1: Expression of VEGFR2 in VEGFR2/NFAT HEK293 Recombinant Cell Line by flow cytometry. VEGFR2/NFAT HEK293 cells (blue) or control NFAT HEK293 cells (red) were stained with PE anti-human CD309 (VEGFR2) Antibody (BioLegend #359903) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

A. Dose response of VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line to human VEGR

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The Assay should include "Stimulated Control", "Cell-Free Control" and "Unstimulated Control" conditions.

Assay Medium: Prepare the Assay Medium using DMEM medium supplemented with 1% (w/vol) protease free BSA (Sigma-Aldrich #A4919)

1. Seed VEGFR2/NFAT Reporter HEK293 cells at a density of ~40,000 cells per well into a white clear-bottom 96-well plate in 100 µl of Thaw Medium 1. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator for ~16 hours.

3. Remove Thaw Medium 1 and add 50 µl of Assay Medium.

4. Incubate the cells at 37°C in a CO_2 incubator for ~1 hour.

5. Add 50 µl of a serial dilution of human VEGF165 protein, prepared at concentrations 2-fold higher than the desired final concentrations in Assay Medium, to the "Stimulated" wells.

6. Add 50 µl of Assay Medium to the "Unstimulated Control" wells.

7. Add 100 µl of Assay Medium to "Cell-Free Control" wells (for determining background luminescence).

8. Incubate the plate at 37°C in a CO_2 incubator for 4 hours.

9. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at Room Temperature (RT) for ~15 to 30 minutes.

10. Measure luminescence using a luminometer.

11. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells.

$$Fold\ induction = \frac{luminescence\ of\ stimulated\ cells - avg.\ background}{avg.\ luminescence\ of\ unstimulated\ cells - avg.\ background}$$

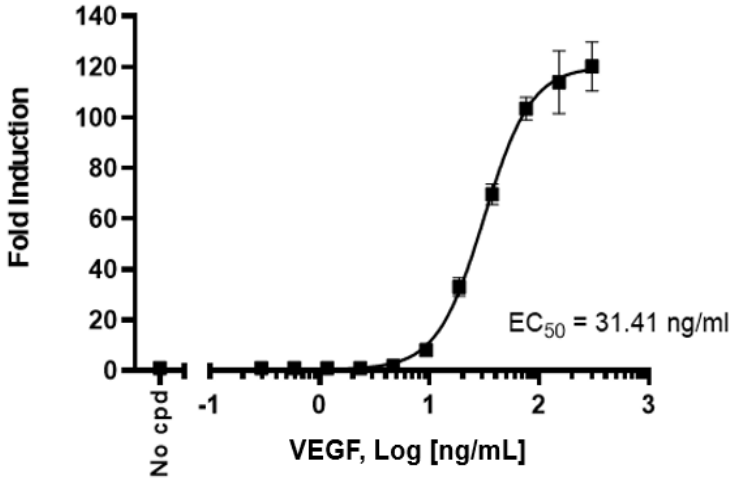

Figure 2: Dose response curve of VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line to VEGF165.
Cells were treated with increasing concentrations of VEGF165 for 4 hours. Luminescence was measured using ONE-Step™ Luciferase Assay System.

B. Inhibition of VEGFR2 kinase activity in VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The Assay should include "No Inhibitor Control", "Cell-Free Control" and "Treated" conditions.

Assay Medium: Prepare the Assay Medium using DMEM medium supplemented with 1% (w/vol) protease free BSA (Sigma-Aldrich #A4919)

1. Seed VEGFR2/NFAT Reporter HEK293 cells at a density of ~40,000 cells per well into a white clear-bottom 96-well plate in 100 µl of Thaw Medium 1. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator for ~16 hours.

3. Remove Thaw Medium 1 and add 50 µl of Assay Medium.

4. Incubate the cells at 37°C in a CO_2 incubator for ~1 hour.

5. Add 25 µl of a serial dilution of VEGFR2 inhibitor, prepared at concentrations 4-fold higher than the desired final concentration in Assay Medium, to the "Treated" wells.

6. Add 25 µl of Assay Medium containing the same concentration of DMSO to the "No Inhibitor Control" wells.

7. Incubate the cells at 37°C in a CO_2 incubator for ~1 hour.

8. Add 25 µl of VEGF 165 to the wells, prepared at 240 ng/ml in the Assay Medium. The final concentration will be 60 ng/ml.

9. Add 100 µl of Assay Medium to "Cell-Free Control" wells (for determining background luminescence).

10. Incubate the plate at 37°C in a CO_2 incubator for 4 hours.

11. Add 100 µl of the ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes.

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent inhibition of luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{(luminescence\ of\ stimulated\ cells - avg.\ background)}{(avg.\ luminescence\ of\ unstimulated\ cells - avg.\ background)}\right) x100$$

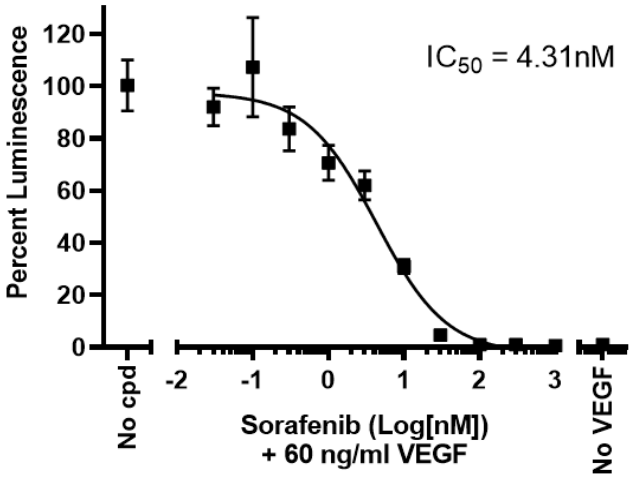

Figure 3. Dose response curve of VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line to Sorafenib.
Cells were treated with increasing concentrations of Sorafenib for 1 hour before stimulation with 240 ng/ml VEGF for 4 hours. Luminescence was measured using ONE-Step™ Luciferase Assay System.

C. Blockade of VEGF binding to VEGFR in VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line by anti-VEGF antibodies

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The Assay should include "Cell-Free Control", "No VEGF, No Antibody Control", "With VEGF, No Antibody Control" and "Test" conditions.

Assay Medium: Prepare the Assay Medium using DMEM medium supplemented with 1% (w/vol) protease free BSA (Sigma-Aldrich #A4919)

1. Seed VEGFR2/NFAT Reporter HEK293 cells at a density of ~40,000 cells per well into a white clear-bottom 96-well plate in 100 µl of Thaw Medium 1. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).

2. Incubate the cells at 37°C in a CO_2 incubator for ~16 hours.

3. Remove Thaw Medium 1 and add 50 µl of Assay Medium.

4. Incubate the cells at 37°C in a CO_2 incubator for ~1 hour.

5. While the cells are incubating, prepare a serial dilution of the anti-VEGF antibody at concentrations 4-fold higher than the desired final concentration in Assay Medium (25 µl/well).

6. Add 25 µl of diluted anti-VEGF antibody to the "Test" wells of a new 96-well plate (not containing the cells) (antibody + VEGF plate).

7. Add 50 µl of Assay Medium to the "No VEGF, No antibody Control" wells in the antibody + VEGF plate.

8. Prepare a solution of VEGF 165 protein at 240 ng/ml in Assay Medium (25 µl/well).

9. Add 25 µl of VEGF 165 to the wells containing the anti-VEGF antibody ("Test" wells) in the antibody + VEGF plate.

10. Add 25 µl of VEGF 165 to the wells labeled "With VEGF, No antibody Control" wells in the antibody + VEGF plate.

11. Incubate the antibody/VEGF plate at 37°C in a CO_2 incubator for ~1 hour.

12. Remove the cells and antibody + VEGF plates from the incubator.

13. Transfer 50 µl of each well of the antibody + VEGF plate to the corresponding wells of the plate containing the cells. The final concentration of VEGF is 60 ng/ml and the final volume is 100 µl.

14. Add 100 µl of Assay Medium to "Cell-Free Control" wells (for determining background luminescence).

15. Incubate the plate at 37°C in a CO_2 incubator for 4 hours.

16. Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes.

17. Measure luminescence using a luminometer.

18. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent inhibition of luciferase reporter expression is the background-subtracted luminescence of the stimulated well divided by the average background-subtracted luminescence of the unstimulated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{(luminescence\ of\ stimulated\ cells - avg.\ background)}{(avg.\ luminescence\ of\ unstimulated\ cells - avg.\ background)}\right) x100$$

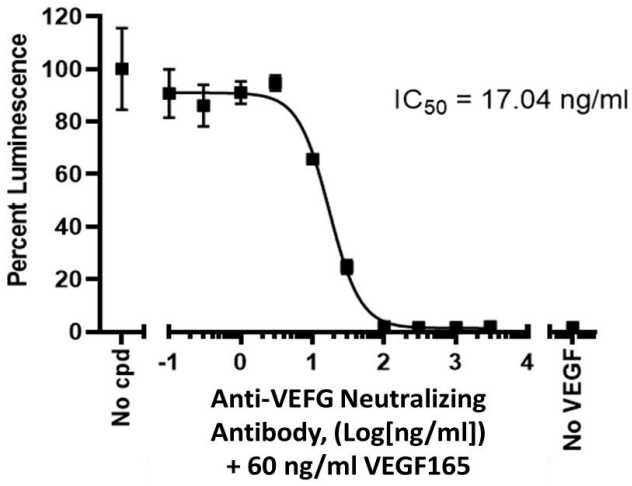

Figure 4. Dose response curve of VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line to Anti-VEGF Neutralizing Antibody.
Increasing concentrations of Anti-VEGF Neutralizing Antibody were pre-incubated with VEGF 165 for 1 hour prior to adding the mix to the cells for 4 hours. Luminescence was measured using the ONE-Step™ Luciferase Assay System.

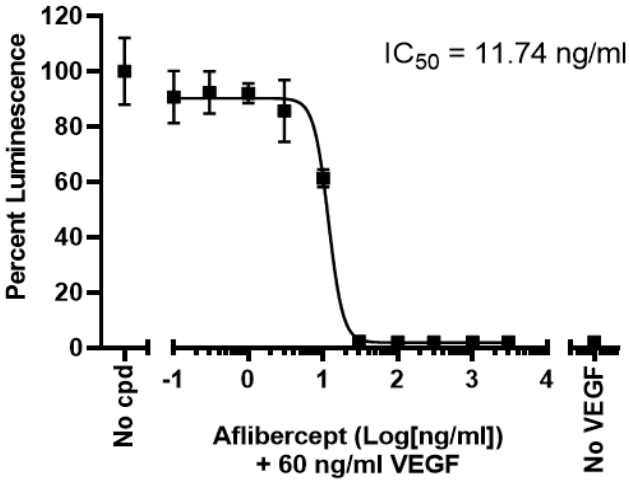

Figure 5. Dose response of VEGFR2/NFAT Reporter HEK293 Recombinant Cell Line to Aflibercept.
Increasing concentrations of Aflibercept were pre-incubated with VEGF 165 for 1 hour prior to adding the mix to the cells for 4 hours. Luminescence was measured using the ONE-Step™ Luciferase Assay System.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence

Human VEGFR2 sequence (accession number NM_002253.1)

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQSGSEQRVEVTECSDGLFC
KTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHGVVYITENKNKTVVIPCLGSISNLNVSLCARYPEKRFVPDG
NRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMYIVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWE
YPSSKHQHKKLVNRDLKTQSGSEMKKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGE
RVRIPAKYLGYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGEKSLISPVDSY
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AANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVSLWCTADRSTFENLTWYKLGPQPLPIHVGELPTPVCKN
LDTLWKLNATMFSNSTNDILIMELKNASLQDQGDYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTAS
GNPPPQIMWFKDNETLVEDSGIVLKDGNRNLTIRRVRKEDEGLYTCQACSVLGCAKVEAFFIIEGAQEKTNLEIIILVGTAVIAMFF
WLLLVIILRTVKRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGIDKTATCRTV
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VGAIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDLYKDFLTLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKNV
VKICDFGLARDIYKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRA
PDYTTPEMYQTMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEEVCDPKF
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NQTSGYQSGYHSDDTDTTVYSSEEAELLKLIEIGVQTGSTAQILQPDSGTTLSSPPV

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
VEGF165, Human (CHO-derived) Recombinant	91006	5 µg/25 µg
Mouse VEGF165 Recombinant	91000	10 µg
VEGF121, Human (CHO-derived) Recombinant	91005	10 µg
VEGFR2 (KDR) Kinase Assay Kit	40325	96 reactions
VEGFR2 (KDR), GST-tag Recombinant	40301	10 µg
VEGFR3 (FLT4), GST-tag Recombinant	40302	10 µg
FLT1, His-tag Recombinant	40223	10 µg
Sorafenib Tosylate	27014	100 mg

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