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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

VEGFR2 / NFAT Reporter – HEK293 Recombinant Cell Line

Catalog #: 79387

Product Description

Recombinant HEK-293 cells expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of human VEGFR2 (Human Vascular Endothelial Growth Factor Receptor 2; KDR, FLK1; ref seq. NM_002253.1).

Background

VEGF (Vascular Endothelial Growth Factor)/VEGFR (Vascular Endothelial Growth Factor Receptor) interaction is an important mediator of angiogenesis as well as cell survival and proliferation. VEGFR2 is a tyrosine receptor 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

- Screen for inhibitors of VEGF/VEGFR2 signaling in a cellular context
- Screen for inhibitors of kinase activity of VEGFR2 in a cellular context

Format

Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone, #SH30024.01) supplemented with 10% FBS (Invitrogen, #26140-079), 1% non-essential amino acids (Hyclone, #SH30238.01), 1 mM Na pyruvate (Hyclone, #SH30239.01), 1% Penicillin/Streptomycin (Hyclone, SV30010.01)

Complete Growth Medium: Thaw Medium 1 (BPS Bioscience, #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031) and 100 µg/ml of Hygromycin B (Life Technologies, #10687-010).

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Cells should be grown at 37°C with 5% CO_2 using complete growth medium.

Assay Medium: DMEM medium (Hyclone #SH30243.01) plus 0.1% BSA.

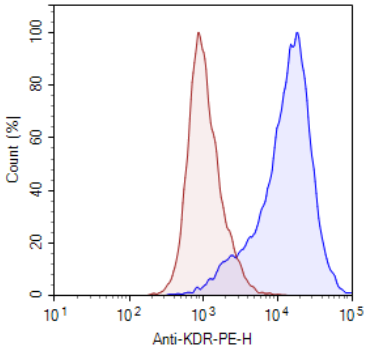
To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional ~3 ml of Thaw Medium 1 (**no Geneticin and Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach ~2.5 x 10^6 cells/ml. At first passage, switch to complete growth medium **(contains Geneticin and Hygromycin B).**

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: 1:5 to 1:10 twice a week.

Note: Just after thawing, the cells may grow at a slower rate. It is recommended to split the cells at no less than 0.4 x 10^6 cells/ml at the beginning of culturing. After ~two passages, the cell growth rate increases and the cells can be split to 0.2 x 10^6 cells/ml.

To freeze down the cells, spin down cells, and resuspend cell pellet in 4°C Freezing Medium (10% DMSO + 90% FBS) at 2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.
It is recommended to expand the cells and at an early passage, freeze down at least 10 vials of cells for future use.

Functional Validation and Assay Performance
VEGFR2/NFAT-HEK293 cells (blue) or control NFAT-HEK293 cells (red) were stained with PE-labeled Anti-VEGFR2(KDR) Antibody (BioLegend, #359903) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



	Samples	Subset	Cell Count
	VEGFR2/NFAT-HEK293 Cell	Live Singlet	13,129
	Control NFAT-HEK293 Cell	Live Singlet	12,459

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The following assays are designed for 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- LiCl (Sigma-Aldrich, #L7026)
- Human VEGF 165 (BPS Bioscience, #91001)
- Media: Thaw Medium 1 (BPS Bioscience, #60187), Complete Growth Medium (see above), Assay medium (see above)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ Luciferase Assay System (BPS Bioscience, #60690)
- Luminometer

A. Dose response of VEGFR2/NFAT Reporter-HEK293 cells to human VEGF

1. Harvest VEGFR2/NFAT Reporter-HEK293 cells from culture in the complete growth medium and seed cells at a density of ~40,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of the complete growth medium.
2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.
3. Remove the complete growth medium and add 90 µl of assay medium.
4. Incubate cells at 37°C in a CO_2 incubator for ~ 1 hour.
5. Add 10 µl of two fold serial dilution of human VEGF 165 protein in assay medium to stimulated wells. Add 10 µl of assay medium to the unstimulated control wells. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate. Incubate the plate at 37°C in a CO_2 incubator for 4 hours.
6. Perform luciferase assay using ONE-Step Luciferase Assay buffer, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.
7. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

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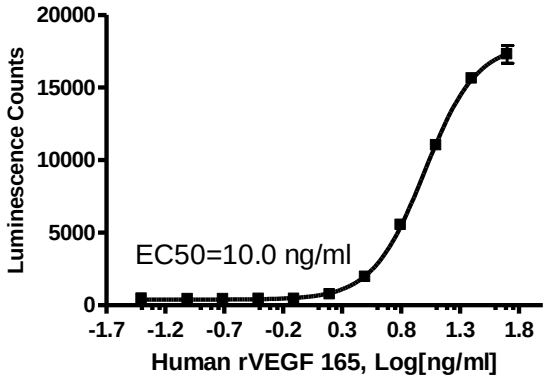
Example: **Dose response of VEGFR2/NFAT-HEK293 cells to human VEGF**



B. Inhibition of kinase activity of VEGFR2 in VEGFR2/NFAT Reporter-HEK293 cells

1. Harvest VEGFR2/NFAT Reporter-HEK293 cells from culture in the complete growth medium and seed cells at a density of ~40,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of the complete growth medium.
2. Incubate cells at 37°C in a CO_2 incubator for ~ 16 hours.
   Remove the complete growth medium and add 80 µl of assay medium.
3. Incubate cells at 37°C in a CO_2 incubator for ~ 1 hours.
4. Add 10 µl of VEGFR2 inhibitor or vehicle control in 10 µl assay medium to the wells. Incubate cells at 37°C in a CO_2 incubator for ~ 1 hours.
5. Add 10 µl of human VEGF 165 protein at final concentration of EC25 in the assay medium to the wells. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.
6. Incubate the plate at 37°C in a CO_2 incubator for 4 hours.
7. Perform luciferase assay using ONE-Step™ Luciferase Assay kit, according to the recommended instructions: Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.
8. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

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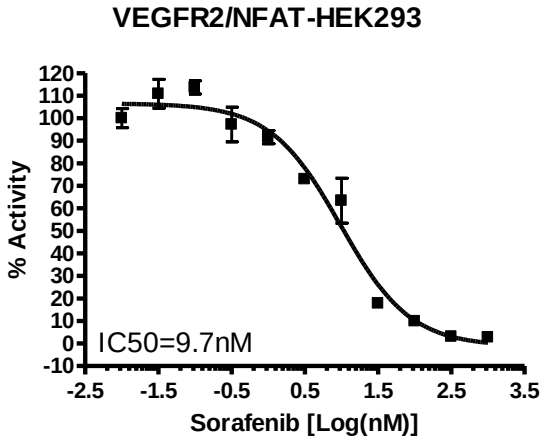
Example: **Inhibition of VEGFR2 by Sorafenib in VEGFR2/NFAT-HEK293 cells**



Sequence

Human VEGFR2 sequence (accession number NM_002253.1)

MQSKVLLAVALWLCVETRAASVGLPSVSLDLPRLSIQKDILTIKANTTLQITCRGQRDLDWLWPNNQSGSE
QRVEVTECSDGLFCKTLTIPKVIGNDTGAYKCFYRETDLASVIYVYVQDYRSPFIASVSDQHGVVYITENK
NKTVVIPCLGSISNLNVSLCARYPEKRFVPDGNRISWDSKKGFTIPSYMISYAGMVFCEAKINDESYQSIMY
IVVVVGYRIYDVVLSPSHGIELSVGEKLVLNCTARTELNVGIDFNWEYPSSKHQHKKLVNRDLKTQSGSEM
KKFLSTLTIDGVTRSDQGLYTCAASSGLMTKKNSTFVRVHEKPFVAFGSGMESLVEATVGERVRIPAKYL
GYPPPEIKWYKNGIPLESNHTIKAGHVLTIMEVSERDTGNYTVILTNPISKEKQSHVVSLVVYVPPQIGEKS
LISPVDSYQYGTTQTLTCTVYAIPPPHHIHWYWQLEEECANEPSQAVSVTNPYPCEEWRSVEDFQGGNKI
EVNKNQFALIEGKNKTVSTLVIQAANVSALYKCEAVNKVGRGERVISFHVTRGPEITLQPDMQPTEQESVS
LWCTADRSTFENLTWYKLGPQPLPIHVGELPTPVCKNLDTLWKLNATMFSNSTNDILIMELKNASLQDQG
DYVCLAQDRKTKKRHCVVRQLTVLERVAPTITGNLENQTTSIGESIEVSCTASGNPPPQIMWFKDNETLVE
DSGIVLKDGNRNLTIRRVRKEDEGLYTCQACSVLGCAKVEAFFIIEGAQEKTNLEIIILVGTAVIAMFFWLLL
VIILRTVKRANGGELKTGYLSIVMDPDELPLDEHCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADA
FGIDKTATCRTVAVKMLKEGATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKFGNLS
TYLRSKRNEFVPYKTKGARFRQGKDYVGAIPVDLKRRLDSITSSQSSASSGFVEEKSLSDVEEEEAPEDL
YKDFLTLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDIYKDPDYVRKGDARL
PLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRRLKEGTRMRAPDYTTPEMYQ
TMLDCWHGEPSQRPTFSELVEHLGNLLQANAQQDGKDYIVLPISETLSMEEDSGLSLPTSPVSCMEEEE
VCDPKFHYDNTAGISQYLQNSKRKSRPVSVKTFEDIPLEEPEVKVIPDDNQTDSGMVLASEELKTLEDRTK
LSPSFGGMVPSKSRESVASEGSNQTSGYQSGYHSDDTDTTVYSSEEAELLKLIEIGVQTGSTAQILQPDS
GTTLSSPPV

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Related Products

Product	Cat. #	Size
VEGF165, Human (Sf9-derived)	91001-1	10 µg
VEGF165, Human (Sf9-derived)	91001-2	100 µg
VEGF165, Human (CHO-derived)	91006-1	5 µg
VEGF165, Human (CHO-derived)	91006-2	25 µg
Mouse VEGF165	91000-1	10 µg
VEGF121, Human (CHO-derived)	91005-1	10 µg
VEGFR2 (KDR) Kinase Assay Kit	40325	96 rxns.
VEGFR2 (KDR), GST-tag	40301	10 µg
VEGFR3 (Flt4), GST-tag	40302	10 µg
VEGFR1 (Flt1), GST-tag	40223	10 µg
Thaw Medium 1	60187	100 ml
Sorafenib Tosylate	27014	100 mg

Notes

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