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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The VEGFR1:VEGF165 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is an ELISA designed for screening and profiling molecules that block the binding between VEGFR1 (vascular endothelial growth factor receptor 1, also known as fms related receptor tyrosine kinase 1) and VEGF165 (vascular endothelial growth factor 165). This kit comes in a convenient 96-well format, with enough recombinant biotin-labeled VEGF165 (amino acids 27-191(end)), purified VEGFR1 (amino acids 27-756), streptavidin-labeled HRP, and assay buffer for 100 binding reactions.

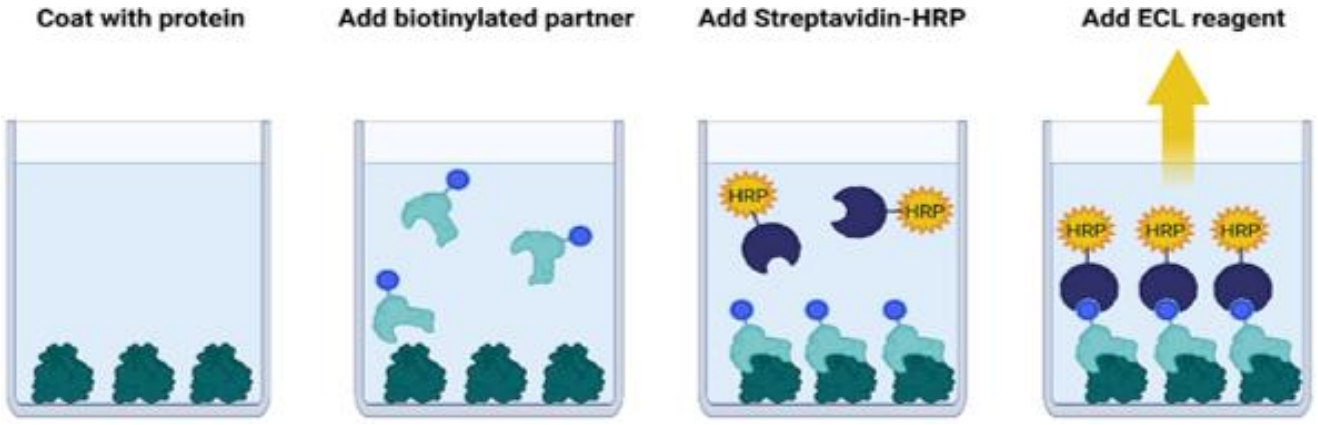

Figure 1: Illustration of the mechanism of VEGFR1: VEGF165 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

A 96-well plate is coated with VEGFR1 protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-VEGF165, the plate is washed and Streptavidin-HRP is added. The ELISA ECL substrate is added, and the resulting signal can be measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of VEGFR1 to VEGF165.

Background

VEGF165 (Vascular Endothelial Growth Factor 165), a potent isoform of VEGF-A, belongs to the VEGF family of homodimer glycoproteins and is produced and secreted by various cells when angiogenesis is required. Angiogenesis involves endothelial cell proliferation, migration, and formation of blood vessels, which under normal conditions serve to provide nutrients and oxygen to tissues during development or wound healing. However, tumor cells can promote new blood vessel formation by secreting pro-angiogenesis factors. VEGF-A can bind to both VEGFR1 (Vascular Endothelial Growth Factor Receptor 1) and VEGFR2, also known as KDR (kinase insert domain receptor), on the surface of endothelial cells or cancer cells. VEGFR2 is considered the main signaling receptor, while VEGFR1 leads to a weak signaling and can be seen as a decoy receptor. Ligand binding induces VEGFR2 receptor dimerization and activates its tyrosine kinase activity. As a result, multiple downstream signaling cascades, including the MAPK (mitogen activated protein kinase) pathway, get activated. The VEGF-VEGFR signal pathway has been a significant target in therapeutic strategies aimed at controlling angiogenesis in diseases like cancer and AMD (age macular degeneration), and several small molecules, neutralizing antibodies and blockers have been FDA-approved. However, the development of drug resistance is still a challenge. The use of combinatory therapy or development of new generation drugs will continue to benefit cancer therapy.

Application(s)

Screen or titrate small molecule inhibitors or antibodies that block VEGFR1 binding to VEGF165 for drug discovery and high-throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102109	VEGFR1, Fc-Avi-Tag *	10 µg	-80°C
102245	VEGF165, His-Avi-Tag, Biotin-Labeled*	3 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrates A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrates B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The initial concentration of both VEGFR1 and VEGF165 is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 1x PBS buffer (Phosphate Buffer Saline)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The DMSO concentration in the final reaction should be ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Non-Coated Condition”, “Blank”, “Positive Control” and “Test Inhibitor” wells.
- We recommend preincubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.
- We recommend using VEGF Blocker (#102019) as internal control. If not running a dose response curve, we recommend running the antibody at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1 - Plate coating with VEGFR1 protein

Coat the plate one day prior to running your samples in the assay test.

1. Thaw **VEGFR1** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **VEGFR1** protein to 2 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **VEGFR1** protein solution to each well.
4. Add 50 µl of PBS to "Non-Coated Condition" wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Immuno Buffer** by diluting 3-fold **3x Immuno Buffer** with distilled water.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
9. Tap the plate onto clean paper towel to remove the liquid.
10. Add 100 µl of Blocking Buffer 2 to every well.
11. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
12. Tap the plate onto clean paper towel to remove the liquid.
13. Start your testing immediately.

Step 2.1: Assessment of the inhibition/blocking of VEGFR1 binding to VEGF165 by an anti-VEGF antibody or blocker.

1. Prepare a serial dilution of **anti-VEGF** antibody or blocker in Blocking Buffer 2 at the desired concentrations (50 µl/well).
2. Add 50 µl of the diluted antibody to the "Test Inhibitor" wells.
3. Add 100 µl of Blocking Buffer 2 to the "Blank" wells.
4. Add 50 µl of Blocking Buffer 2 to the "Positive Control" wells.
5. Incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.
6. Thaw the **Biotin-VEGF165** on ice. Briefly spin the tube to recover the full content.

7. Dilute **Biotin-VEGF165** to 0.5 µg/ml in Blocking Buffer 2 (50 µl/well).

8. Add 50 µl of diluted **Biotin-VEGF165** to the "Test Inhibitor" and "Positive Control" wells.

9. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	Positive Control	Test Inhibitor
Blocking Buffer 2	100 µl	50 µl	-
Test Inhibitor	-	-	50 µl
Diluted Biotin-VEGF165 (0.5 µg/ml)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

10. Wash the plate three times with 1x Immuno Buffer 1.

11. Block the wells by adding 100 µl of Blocking Buffer 2 to every well and incubate for 10 minutes.

12. Tap the plate onto clean paper towel to remove the liquid.

Step 3.1: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (100 µl/well).

2. Add 100 µl of the diluted Streptavidin-HRP to each well.

3. Incubate the plate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.

5. Tap the plate onto clean paper towel to remove the liquid.

6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

7. Add 100 µl of mix to each well.

8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

9. The "Blank" value should be subtracted from all readings.

Step 2.2: Assessment of the inhibition/blocking of VEGFR1 binding to VEGFR165 by small molecules.

1. Prepare the test inhibitor (5 µl/well): For a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   1.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water at concentrations 10-fold higher than the desired final concentrations. Distilled water is the Diluent Solution.

   OR

1.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

Using distilled water containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO in the assay should not exceed 1%.

2. Add 5 µl of diluted Test Inhibitor to each well labeled "Test Inhibitor".
3. Add 5 µl of the Diluent Solution to the "Positive Control" and "Blank" wells.
4. Thaw **Biotin-VEGF165** on ice. Briefly spin the tube to recover the full content.
5. Dilute Biotin-VEGF165 to 0.5 µg/ml in Blocking Buffer 2 (20 µl/well).
6. Add 20 µl of diluted Biotin-VEGF165 to the wells labeled "Test Inhibitor" and "Positive Control".
7. Add 25 µl of Blocking Buffer 2 to the "Test Inhibitor" and "Positive Control" wells.
8. Add 45 µl of Blocking Buffer 2 to the "Blank" wells.
9. Incubate the plate at RT for 1 hour with gentle agitation.
10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1.
11. Block the wells by adding 100 µl of Blocking Buffer 2 to every well and incubate for 10 minutes.
12. Tap the plate onto clean paper towel to remove the liquid.

	Blank	**Positive Control**	**Test Inhibitor**
Blocking Buffer 2	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted Biotin-VEGF165 (0.5 µg/ml)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

Step 3.2: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (100 µl/well).
2. Add 100 µl of the diluted Streptavidin-HRP to each well.

3. Incubate the plate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.

5. Tap the plate onto clean paper towel to remove the liquid.

6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

7. Add 100 µl of mix to each well.

8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

9. The "Blank" value should be subtracted from all readings.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

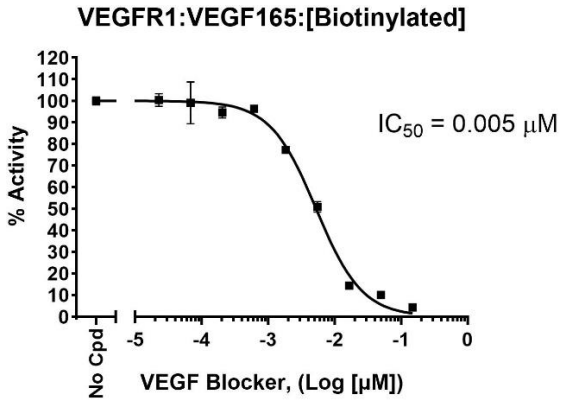

Figure 1. Inhibition of VEGFR1:VEGF165 binding by VEGFR Blocker.
VEGFR1:VEGF165 binding was evaluated in the presence of increasing concentrations of VEGFR Blocker (#102019). Results are expressed as percent activity, in which the binding activity in the absence of inhibitor is set to 100%.

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

Troubleshooting Guide

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

References

Wang L., *et al.*, 2024 *Front Pharmacol.* 14:1307860.

Related Products

Products	*Catalog #*	*Size*
VEGFR Blocker	102019	
VEGF165:VEGFR2[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	82582	96 reactions
Lumi-Verse™ VEGFR2 (KDR) Kinase Assay Kit	78857	96 reactions
VEGFR2/NAFT Reporter HEK293 Recombinant Cell Line	79387	2 vials
VEGFR1, His-Tag Recombinant	40223	10 µg
VEGFR3(FLT4) Kinase Assay Kit	79738	96 reactions

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