VEGFR2 (KDR) Kinase Assay Kit

#40325
96 reactions

Description

The VEGFR2 (KDR) Kinase Assay Kit is designed to measure VEGFR2 (KDR) kinase activity for screening and profiling applications using Kinase-Glo™ MAX as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant VEGFR2 (KDR) kinase (amino acids 805-1356), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Note: For an assay with higher sensitivity, please refer to Lumi-Verse™ VEGFR2 (KDR) Kinase Assay Kit (BPS Bioscience #78857).

Background

Vascular endothelial growth factor receptor 2 (VEGFR2), also called Kinase insert Domain Receptor (KDR), is a tyrosine kinase (TK) receptor for VEGF. EGFR (Epidermal Growth Factor Receptor) overexpression is a common feature in many cancer types, such as breast and ovarian cancer. EGFR activation results in VEGF expression and signaling via VEGFR2, promoting angiogenesis and tumor progression. The inhibition of VEGFR2 via small molecules, antibodies or CAR, alone or in combination with EGFR, are promising therapeutic strategies for cancer immunotherapy.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
40301	VEGFR2 (KDR), GST-Tag*	3 µg	-80°C
79334	5x Kinase Buffer 1	1.5 ml	-20°C
79686	500 µM ATP	100 µl	-20°C
40217	PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml)	100 µl	-20°C
79696	White 96-well plate	1	Room Temperature

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

Name	Catalog #
Kinase-Glo™ MAX Assay	Promega #V6071
DTT (Dithiothreitol), 1 M, optional	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	
30°C incubator	

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.

Assay Principle

The **Kinase-Glo™ MAX Assay (Promega, #V6930)** quantifies the amount of ATP present at the time of the reaction. ATP is used as substrate by the reagent to mono-oxygenate luciferin. The luminescent signal correlates inversely with the kinase activity and it is linear up to 0.5 mM ATP.

Contraindications

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

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw **5x Kinase Buffer 1**, **500 µM ATP** and **PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml).**

   *Optional: If desired, add DTT to **5x Kinase Buffer** 1 to make a 10 mM DTT.*

2. Prepare 3 ml of **1x Kinase Buffer 1** by mixing 600 µl of **5x Kinase Buffer 1** with 2,400 µl of distilled water.

   *Note: Three (3 ml) of **1x Kinase Buffer 1** is sufficient for 100 reactions.*

3. Prepare a **Master Mix** (25 µl/well): N wells x (6 µl of 5x Kinase Buffer 1 + 1 µl of 500 µM ATP + 1 µl of PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) + 17 µl of distilled water).

4. Add 25 µl of Master Mix to every well.

5. 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.

   5.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in the 1x Kinase Buffer 1, 10-fold more concentrated than the desired final concentrations.
   For the positive and negative controls, use 1x Kinase Buffer 1 (Diluent Solution).
   OR
   5.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Kinase Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Buffer 1 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x Kinase Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

6. Add 5 µl of Test Inhibitor to each well labeled “Test Inhibitor.”

7. Add 5 µl of Diluent Solution to the “Blank” and “Positive Control” wells.

8. Add 20 µl of 1x Kinase Buffer 1 to the “Blank” wells.

9. Thaw **VEGFR2 (KDR)** on ice. Briefly spin the tube to recover its full content.

10. Dilute the protein kinase (20 µl/well) to 2 ng/µl using 1x Kinase Buffer 1.

    Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. This kinase is particularly sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use the thawed protein and do not re-use the diluted kinase.

11. Initiate the reaction by adding 20 µl of diluted VEGFR2 Kinase to the wells designated “Positive Control” and "Test Inhibitor."

Component	**Blank**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
1x Kinase Buffer 1	20 µl	-	-
Diluted VEGFR2 (KDR) (2 ng/µl)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

12. Incubate at 30°C for 45 minutes.

13. During the incubation, thaw the Kinase-Glo™ MAX reagent. At the end of the 45-minute reaction, add 50 µl of Kinase-Glo™ MAX reagent to each well. Cover the plate with aluminum foil and incubate at Room Temperature for 15 minutes.

14. Immediately read in a luminometer or a microplate reader capable of reading luminescence.

15. The “Blank” value should be subtracted from all other readings.

Reading Luminescence

Luminescence is the emission of light resulting from a chemical reaction. The detection of luminescence requires no wavelength selection because the method used is emission photometry and not emission spectrophotometry.

To properly read luminescence, 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: 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

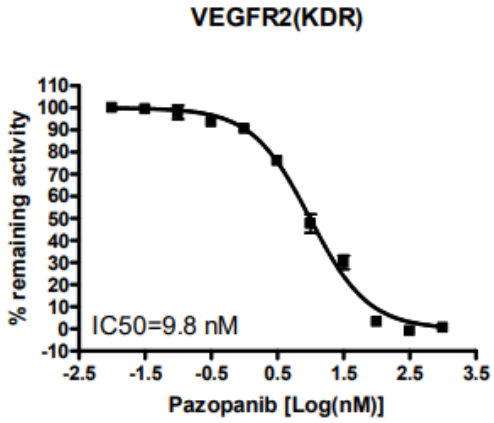

Figure 1. Inhibition of VEGFR2 (KDR) kinase Activity by Pazopanib (SelleckChem #S3012).
The inhibition of VEGFR2 (KDR) kinase activity was measured in the presence of increasing inhibitor concentrations. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

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
Mashima T,*et al.,* 2021 *Scientific Reports* 11: 15125.

Related Products

Products	*Catalog #*	*Size*
VEGFR2 (KDR) Kinase Assay Kit	40325	96 reactions
VEGFR2 (KDR), GST-Tag Recombinant	40301	10 µg
VEGFR2/ NFAT Reporter – HEK293 Cell Line	79387	2 Vials

Version 111324