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Description

The Lumi-Verse™ VEGFR2 (KDR) Kinase Assay Kit is designed to measure Vascular Endothelial Growth Factor Receptor 2 (VEGFR2) tyrosine kinase activity for screening and profiling applications, using the highly sensitive ADP-Glo™ as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant VEGFR2 (KDR) kinase domain (amino acids 805-1356), kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

Vascular endothelial growth factor receptor 2 (VEGFR2), also known as Kinase insert Domain receptor (KDR), is a tyrosine kinase receptor for the growth factor 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 or antibodies, alone or in combination with inhibitors of EGFR, are promising therapeutic strategies for cancer therapy.

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), aa 805-1356, GST-Tag* | 2 µg | -80°C |
| 79334 | 5x Kinase Buffer 1 | 1.5 ml | -20°C |
| 79686 | 500 µM ATP | 50 µl | -20°C |
| 40217 | PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) | 50 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 # |
|---|----------------|
| ADP-Glo™ Kinase Assay | Promega #V6930 |
| 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 **ADP-Glo™ Kinase Assay (Promega #V6930)** quantifies the amount of ADP produced by a kinase upon phosphorylation of a substrate. First, addition of the ADP-Glo™ reagent terminates the reaction and quenches the remaining ATP. Second, the addition of the Kinase Detection reagent converts the produced ADP to ATP. The new ATP is quantified via a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear up to 1 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** (12.5 μl/well): N wells x (6 μl of 5x Kinase Buffer 1 + 0.5 μl of 500 μM ATP + 0.5 μl of PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) + 5.5 μl of distilled water).
4. Add 12.5 μl of Master Mix to every well.
5. Prepare the **Test Inhibitor** (2.5 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 25 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 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 2.5 μ l of Test Inhibitor to each well labeled "Test Inhibitor".
7. Add 2.5 μ l of Diluent Solution to the "Blank" and "Positive Control" wells.
8. Add 10 μ l 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 (10 μ l/well) to 2 ng/ μ l 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.

8. Initiate the reaction by adding 10 μ l of diluted VEGFR2 Kinase to the wells designated "Positive Control" and "Test Inhibitor."

| Component | Blank | Positive Control | Test Inhibitor |
|--------------------------------------|-----------------------------|-----------------------------|-----------------------------|
| Master Mix | 12.5 μ l | 12.5 μ l | 12.5 μ l |
| Test Inhibitor | - | - | 2.5 μ l |
| Diluent Solution | 2.5 μ l | 2.5 μ l | - |
| 1x Kinase Buffer 1 | 10 μ l | - | - |
| Diluted VEGFR2 (KDR) (2 ng/ μ l) | - | 10 μ l | 10 μ l |
| Total | 25 μl | 25 μl | 25 μl |

9. Incubate at 30°C for 45 minutes.
10. During the incubation, thaw the ADP-Glo™ reagent. At the end of the 45-minute reaction, add 25 μ l of ADP-Glo™ reagent to each well. Cover the plate with aluminum foil and incubate at Room Temperature for 45 minutes.
11. Thaw the Kinase Detection Reagent. At the end of the 45-minute incubation, add 50 μ l of Kinase Detection reagent to each well. Cover the plate with aluminum foil and incubate at Room Temperature for another 45 minutes.
12. Immediately read in a luminometer or a microplate reader capable of reading luminescence.
13. The "Blank" value is 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

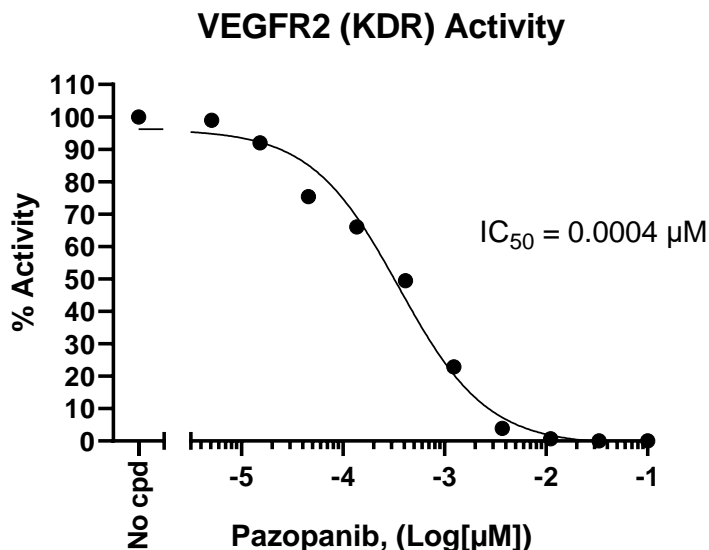


Figure 1: Inhibition of VEGFR2 (KDR) kinase Activity by Pazopanib (SelleckChem #S3012).

The inhibition of VEGFR2 (KDR) tyrosine 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 further questions, please email support@bpsbioscience.com.

References

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

Related Products

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