Description

The CSF1R Kinase Assay Kit is designed to measure CSF1R kinase activity for screening and profiling applications using ADP-Glo® as a detection reagent. The assay kit comes in a convenient 96-well format, with enough purified recombinant CSF1R kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

Colony Stimulating Factor 1 Receptor (CSF1R, also known as CSFR, CD115, and M-CSF-R) is a tyrosine kinase transmembrane receptor activated either by CSF1 (MCSF) or interleukin-34 (IL-34), causing homodimerization and activation of the kinase activity. CSF1R is expressed on the surface of monocytes and macrophages, controlling their growth, survival, or differentiation. A number of tumor cells overexpress the cytokine CSF1, driving the development and survival of Tumor-Associated Macrophages, which in turn suppress the local immune response to the cancer.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
79110	CSF1R (kinase domain)*	30 µg	-80°C
79334	Kinase Assay Buffer 1 (5x)	1.5 ml	-20°C
79686	ATP (500 µM)	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), 1M, 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.

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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, addition of the Kinase Detection reagent converts the produced ADP to ATP. The new ATP is quantified by a luciferase reaction. The luminescent signal correlates with the amount of ADP generated by the kinase and is linear 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 Assay Buffer 1**, (**500 µM) ATP,** and **PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml).**
   *Optional: If desired, add DTT to **5x Kinase Assay Buffer** 1 to make a 10 mM DTT concentration (for example, add 10 µl of 1 M DTT to 1 ml of **5x Kinase Assay Buffer 1**).*

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

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

3. Prepare the **Master Mix** (12.5 µl/well): N wells x (6 µl of **5x Kinase Assay Buffer 1** + 0.5 µl of **ATP (500 µM)** + 0.5 µl of PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) + 5.5 µl of distilled water. Add 12.5 µl to every well.

4. 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.

   If the Test Inhibitor is water-soluble:
   4.1 Prepare serial dilutions in the **1x Kinase Assay Buffer 1**, 10-fold more concentrated than the desired final concentrations.
   4.2 For the positive and negative controls, use **1x Kinase Assay Buffer 1** (Diluent Solution).
   OR
   If the Test inhibitor is soluble in DMSO:
   4.1 Prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in **1x Kinase Assay Buffer 1** to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.
   4.2 Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Kinase Assay Buffer to keep the concentration of DMSO constant.
   4.3 For positive and negative controls, prepare 10% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).
   Note: The final concentration of DMSO should not exceed 1%.

5. Add 2.5 µl of **Test Inhibitor** to each well labeled “Test Inhibitor.” For the “Positive Control" and “Blank,” add 2.5 µl of **Diluent Solution** (either kinase assay buffer or 10% DMSO in kinase assay buffer, as described above).

6. To the wells designated as "Blank," add 10 µl of **1x Kinase Assay Buffer 1**.

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7. Thaw **CSF1R kinase** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase (10 µl/well) to 30 ng/µl using **1x Kinase Assay 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. Do not re-use the thawed protein and do not re-use the diluted kinase.

8. Initiate the reaction by adding 10 µl of diluted Kinase to the wells designated “Positive Control” and "Test Inhibitor."

Component	Blank	Positive Control	Test Inhibitor
Master Mix	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-
1x Kinase Assay Buffer 1	10 µl	-	-
CSF1R (30 ng/µl)	-	10 µl	10 µl
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 µl 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 µl 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. 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).

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Example Results

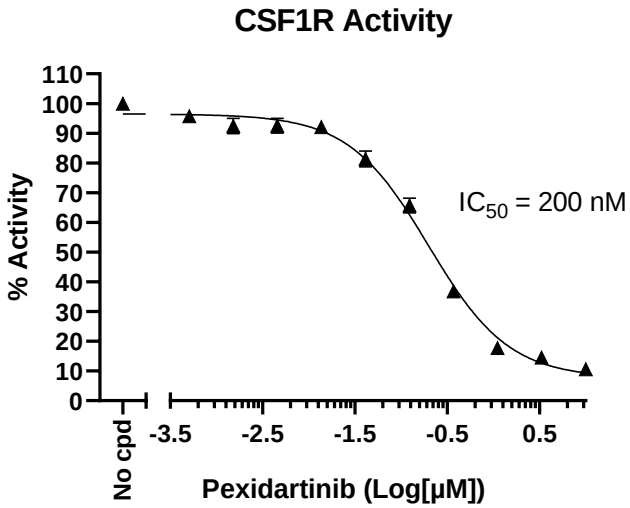

Figure 1: Inhibition of CSF1R kinase Activity by Pexidartinib (Medchemexpress #HY-16749).
The inhibition of CSF1R kinase activity was measured in the presence of increasing inhibitor concentrations. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (kinase activity in the absence of inhibitor, set at 100%).

Results are Representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

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

References

1. Walter, M., *et al*. (2007). *Mol. Biol.* 367 (3): 839-847.
2. Li, J., *et al*. (2006). *Genesis* 44 (7): 328-335.

Related Products

Products	*Catalog #*	*Size*
CSF1R, Fc-Fusion, Avi-Tag, Biotin-Labeled Recombinant	79343	25 µg/50 µg
CSF1R / SRE Reporter Kit (MAPK/ERK Signaling Pathway)	79379	500 reactions
CSF1R / SRE Reporter HEK293 Recombinant Cell Line	79380	2 vials
ONE-Step™ Luciferase Assay System	60690	10 ml/100 ml/500 ml/1 L

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