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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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BTK (C481S) Kinase Assay Kit

#78801
96 reactions

Description

The BTK (C481S) Kinase Assay Kit is designed to measure BTK (C481S) 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 BTK (C481S) kinase, kinase substrate, ATP and kinase assay buffer for 100 enzyme reactions.

Background

Bruton's tyrosine kinase (BTK), is an enzyme that plays a role in the functionality and maturation of B cells. The BTK pathway has implications for a number of autoimmune disorders including isolated growth hormone deficiency type III and rheumatoid arthritis. However, as therapies have progressed, specifically in chronic lymphocytic leukemia (CLL), resistance has become more common in patients where the disease has progressed due to prolonged therapy. A mutation in the BTK 481 cysteine residue to which inhibitors bind covalently is often linked to BTK inhibition (BTKi) in CLL patients where resistance has been led to disease progression. Targeting the BTK mutation of interest (C481S) has shown promising clinical efficacy in treating CLL patients that are no longer responding to common therapy.

Application(s)

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

Supplied Materials

Catalog #	Name	Amount	Storage
101039	BTK (C481S), His-Tag*	10 µg	-80°C
79334	5X Kinase Assay Buffer 1	1.5 ml	-20°C
79686	ATP (500 µM)	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	Ordering Information
ADP-Glo® Kinase Assay	Promega #V6930
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.

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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%.
- DTT may interfere with the assay.

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).**

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)** + 5 µl of PTK Substrate (Poly-Glu,Tyr 4:1) (10 mg/ml) + 1 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**.

7. Thaw **BTK (C481S) kinase** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase (10 µl/well) to 10 ng/µl **[please check units of concentration]** using **1x Kinase Assay Buffer 1**.

Note: 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 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	-	-
BTK (C481S) (10 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).

Example Results

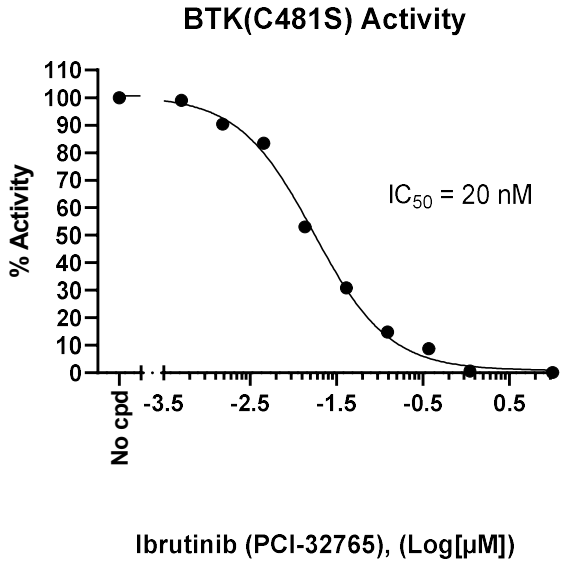

Figure 1: Inhibition of BTK (C481S) kinase Activity.
The inhibition of BTK (C481S) kinase activity was measured in the presence of increasing inhibitor concentrations, Ibrutinib (PCI-32765) (SelleckChem #S2680), using the BTK (C481S) Kinase Assay Kit (BPS Bioscience #78801). The "Blank" value was subtracted 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

1. Rawlings, *et al*. Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. Science (1993). 261(5119): 358–361.
2. Naeem, A., *et al.* Pirtobrutinib Targets BTK C481S in Ibrutinib-Resistant CLL but Second-Site BTK Mutations Lead to Resistance. (2022). Blood Advances.

Related Products

Products	*Catalog #*	*Size*
AXL Kinase Assay Kit	79711	96 reactions
BTK Assay Kit	79568	96 reactions
EPHA4 Kinase Assay Kit	78592	96 reactions
FGFR2 Assay Kit	79804	96 reactions
RET Assay Kit	79566	96 reactions