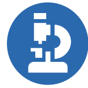
SZABO SCANDIC
Part of Europa Biosite

# Produktinformation

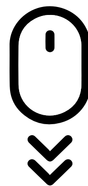
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

**SZABO-SCANDIC HandelsgmbH**
Quellenstraße 110, A-1100 Wien
T. +43(0)1 489 3961-0
F. +43(0)1 489 3961-7
mail@szabo-scandic.com
www.szabo-scandic.com

linkedin.com/company/szaboscandic

## Description

The Chemi-Verse™ Aurora Kinase B Assay Kit is a luminescence assay designed to measure Aurora Kinase B 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 Aurora Kinase B, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

## Background

Aurora Kinase B, also known as serine/threonine-protein kinase 5, is part of the Aurora subfamily. Aurora Kinase B is a CPC (chromosomal passenger complex) protein. It plays a role in microtubule organization during mitosis and meiosis, forming a complex with Survinin, Borealin and INCENP (inner centromere protein) and binding to EB1 (end-binding protein 1). It has a pattern of expression and subcellular localization that varies with the cell cycle stage, in agreement with its role as cell cycle regulator. It has oncogenic activity, being able to lead to the development of cells with abnormal number of chromosomes. Aurora Kinase B also promotes cancer by decreasing expression of p21, hyperactivating CDK1 (cyclin-dependent kinase 1) and regulating class IIa HDACs (histone deacetylase). Interestingly, the use of Aurora inhibitors can result in an increase in the number of chromosome alterations in cancer cells, resulting in cell death, and can also decrease expression of cyclin B1 and D1 and elevate caspase 3 levels in lymphoma cells. Further studies will elucidate the exact mode of action of Aurora Kinase B in cancer development and progression and open new therapeutic avenues.

## Applications

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

## Supplied Materials

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 40002 | Aurora Kinase B GST-Tag, His-Tag* | 20 µg | -80°C |
| 79334 | 5x Kinase Buffer 1 | 1.5 ml | -20°C |
| 79686 | 500 µM ATP | 50 µl | -20°C |
| 78514 | Myelin basic protein (MBP), 5 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 | Ordering Information |
|---|---|
| ADP-Glo™ Kinase Assay | Promega #V6930 |
| DTT (Dithiothreitol) 1M | |
| 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 generated 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.
- The assay should include “Blank”, “Positive Control” and “Test Inhibitor” conditions.

1. Thaw **5x Kinase Assay Buffer 1**, **500 µM ATP,** and **MBP (5 mg/ml).**

   *Optional: Make 5x Kinase Assay Buffer 1 with 100 µM DTT.*

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

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

3. Prepare a **Master Mix** (12.5 µl/well): N wells x (6 µl of 5x Kinase Assay Buffer 1 + 0.5 µl of 500 µM ATP + 0.5 µl of MBP (5 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 Assay Buffer 1**, 10-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use **1x Kinase Assay Buffer 1** (Diluent Solution).



BPS Bioscience

Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

***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 Assay 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 Assay Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Kinase Assay 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 µl of **Test Inhibitor** to each well labeled "Test Inhibitor".
7. Add 2.5 µl of **Diluent Solution** to the "Positive Control" and "Blank" wells.
8. Add 10 µl of **1x Kinase Assay Buffer 1** to the "Blank" wells.
9. Thaw **Aurora Kinase B** on ice. Briefly spin the tube to recover its full content.
10. Dilute the protein kinase (10 µl/well) to 20 ng/µl with **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. 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".
9. Incubate at 30°C for 45 minutes.
10. Thaw the ADP-Glo™ reagent.
11. At the end of the 45-minute reaction, add 25 µl of ADP-Glo™ reagent to each well.
12. Cover the plate with aluminum foil and incubate at Room Temperature (RT) for 45 minutes.
13. Thaw the Kinase Detection Reagent.
14. Add 50 µl of Kinase Detection reagent to each well. Cover the plate with aluminum foil and incubate at RT for another 45 minutes.
15. Immediately read in a luminometer or a microplate reader capable of reading luminescence.



BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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

| 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 | - | - |
| Diluted Aurora Kinase B (20 ng/µl) | - | 10 µl | 10 µl |
| **Total** | **25 µl** | **25 µl** | **25 µl** |

**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**

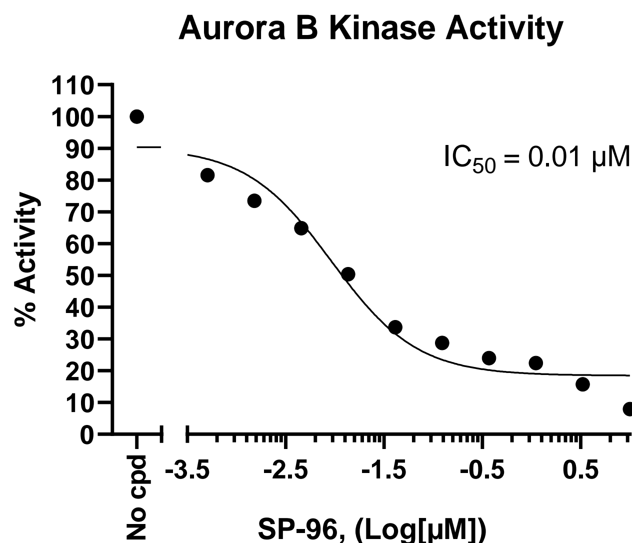



*Figure 1: Inhibition of Aurora Kinase B activity by SP-96.*

The inhibition of Aurora B kinase activity was measured in the presence of increasing concentrations of SP-96 (SelleckChem #S9658). 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%).

*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**

Tang A., *et al.*, 2017 *Oncotarget* 8(14):23937-23954.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| Chemi-Verse™ Aurora Kinase A Assay Kit | 82095 | 96 reactions |
| Chemi-Verse™ Aurora Kinase C Assay Kit | 82096 | 96 reactions |
| Aurora Kinase A, His-Tag (HEK-293-derived) Recombinant | 100112 | 10 µg |
| Aurora Kinase A, His-Tag (Sf9-derived) Recombinant | 40004 | 10 µg |
| Aurora Kinase C, GST-tag Recombinant | 40178 | 10 µg |



BPS Bioscience
Our products are for research use only, not for diagnostic or therapeutic use
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com