

Description

The DYRK1B Kinase Assay Kit is designed to measure DYRK1B 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 DYRK1B kinase, kinase substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

Background

DYRK1B is a dual-specificity kinase with serine/threonine and tyrosine kinase activities. It is activated as part of the cellular response to DNA damage and regulates gene transcription, notably by increasing the activity of transcription factors such as TCF1 or FOXO1. DYRK1B may play a role in several pathologies as a result of its metabolic activities. It also represents a potential therapeutic target in several types of cancer [1].

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
100646	DYRK1B, GST-tag*	3 µg	-80°C
79334	Kinase assay buffer 1 (5x)	1.5 ml	-20°C
79686	ATP (500 µM)	100 µl	-20°C
78393	DYRK-tide (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	Promega #V6071
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.

Assay Principle

Kinase activity is measured using **Kinase-Glo™ Max** (Promega, #V6071). The addition of the reagent results in the generation of a luminescent signal that correlates with the amount of ATP. The reagent is linear to 100 μM 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**, **ATP** and **DYRK-tide (10 mg/ml) substrate**.
Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM DTT concentration (for example, add 10 μl of 1 M DTT to 1 ml of **5x Kinase assay buffer**).
2. Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 μl of **5x Kinase assay buffer** with 2400 μl water. Three (3) ml of **1x Kinase assay buffer** is sufficient for 100 reactions.
3. Prepare the Master Mix (25 μl/well): N wells x (5 μl of **5x Kinase assay buffer** + 1 μl of **ATP (500 μM)** + 1 μl of **DYRK-tide (10 mg/ml)** + 18 μl of distilled water). Add 25 μl to every well.
4. 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.
 - a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x Kinase Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Kinase Assay Buffer (Diluent Solution).
 - b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Kinase Assay Buffer 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 to keep the concentration of DMSO constant.

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

The final concentration of DMSO should not exceed 1%.

- Add 5 μl of Test Inhibitor to each well labeled "Test Inhibitor." For the "Positive Control" and "Blank," add Diluent Solution (either distilled water or 10% DMSO in water, as described above).
- To the wells designated as "Blank," add 20 μl of **1x Kinase assay buffer**.
- Thaw **DYRK1B kinase** on ice. Briefly spin the tube to recover its full contents. Dilute the protein kinase (20 μl /well) to 1.25 ng/ μl using **1x Kinase assay buffer**.

Notes: 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.

- Initiate the reaction by adding 20 μl of diluted 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	20 μl	-	-
DYRK1B (1.25 ng/ μl)	-	20 μl	20 μl
Total	50 μl	50 μl	50 μl

- Incubate at 30°C for 45 minutes.
- 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 the plate at room temperature for 15 minutes.
- 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

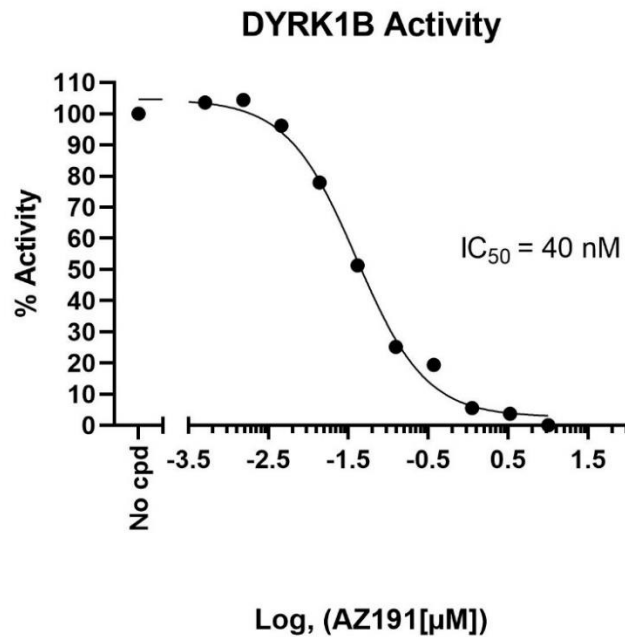


Figure 1: Inhibition of DYRK1B kinase Activity by AZ191. The inhibition of DYRK1B kinase activity was measured in the presence of increasing inhibitor concentrations using the DYRK1B Kinase Assay Kit (BPS Bioscience #78401). The Blank value was subtracted from all other values. Results are expressed as 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. Becker W. A wake-up call to quiescent cancer cells - potential use of DYRK1B inhibitors in cancer therapy. *FEBS J.* 2018; **285(7)**: 1203-1211.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
DYRK2 Kinase Assay Kit	78392	96 reactions
DYRK1B, GST-tag	100646	10 μg
DYRK2, His-Tag	40053	10 μg
TTK (MPS-1) Kinase Assay Kit	78356	96 reactions
TTK, GST-tag	40291	10 μg