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Data Sheet

AKT1 Assay Kit

Catalog # 78038
96 Reactions

BACKGROUND: AKT1, also known as Protein Kinase B Alpha (PKBα), is a serine/threonine kinase found in various cell types throughout the body, where it plays a critical role in regulating cell survival, insulin signaling, angiogenesis and tumor formation as well as controlling apoptosis. Importantly, the signaling pathway involving AKT1 kinase appears to be essential for the normal development and function of the nervous system. Dysregulation of Akt1 contributes to a number of serious diseases, including cancer, diabetes, cardiovascular and neurological diseases.

DESCRIPTION: The *AKT1 Assay Kit* is designed to measure AKT1 activity for screening and profiling applications using Kinase-Glo® Kinase Assay as a detection reagent. The *AKT1 Assay Kit* comes in a convenient 96-well format, with enough purified recombinant AKT1 enzyme, AKT1 Substrate, ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40003	AKT1	10 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer 1	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
	AKT1 Substrate (5 mg/ml)	100 µl	-20°C	
79696	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo® Kinase Assay (Promega #V6071)
Dithiothreitol (DTT, 0.5 M)
Microplate reader capable of reading luminescence
Adjustable micropipettor and sterile tips
30°C incubator

APPLICATIONS: Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: Up to 6 months when stored as recommended.

REFERENCES:
1. Kuijl C, *et al., Nature*. 2007 Nov 29; **450 (7170):**725-30.
2. Vogiatzi, P. and Giordano, A. *Cancer Biol Ther.* 2007 Aug 3; **6(10):**1521-1524.

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ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Thaw **5x Kinase assay buffer**, **ATP (500 µM),** and **AKT1 Substrate**.

2) Prepare the master mixture (25 µl per well): N wells x (6 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 1 µl **AKT1 Substrate** + 17 µl distilled water. Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	6 µl	6 µl	6 µl
ATP (500 µM)	1 µl	1 µl	1 µl
AKT1 Substrate	1 µl	1 µl	1 µl
Water	17 µl	17 µl	17 µl
Test Inhibitor	–	5 µl	–
Inhibitor Buffer (no inhibitor)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
AKT1 (5 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

1) Prepare 10X concentrated inhibitor in an aqueous-based solution. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease the enzyme activity. For example, to test an inhibitor at 10 µM, dilute 1 mM inhibitor in 100% DMSO with water to make a 100 µM inhibitor in 10% DMSO (aq). Then, add 5 µl of the 100 µM solution to the assay to make a 10 µM in 1% DMSO concentration in the final reaction mixture.*

3) Add 5 µl of Inhibitor solution of each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of the same solution without inhibitor (Inhibitor buffer, i.e. 10% DMSO(aq)). Be sure to maintain the same concentration of DMSO in the Positive Control sample as the Test sample.

2) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2,376 µl distilled water and 24 µl of 0.5M DTT. 3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.

3) To the wells designated as "Blank," add 20 µl of **1x Kinase assay buffer**.

4) Thaw **AKT1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **AKT1** required for the assay and

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dilute enzyme to 5 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C.

Note: AKT1 enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

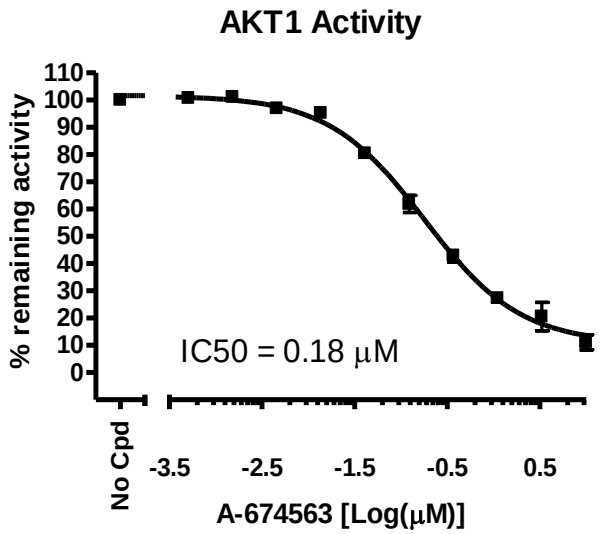
5) Initiate reaction by adding 20 µl of diluted **AKT1** to the wells designated "Positive Control" and "Test Inhibitor." Incubate at 30˚C for 45 minutes.

6) Thaw Kinase-Glo Max reagent.

7) After the 45 minute reaction, add 50 µl of Kinase-Glo Max reagent to each well. Cover plate with aluminum foil and incubate the plate at room temperature for 15 minutes.

8) Measure luminescence using the microplate reader. Value of "Blank" reading should be subtracted from all other measurements.

Example of Assay Results:



Inhibition of AKT1 by A-674563, measured using the *AKT1 assay kit* (BPS Bioscience #78038). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
AKT1, His-tag	40003	10 µg
5x Kinase buffer 1	79334	10 ml
ATP (500 µM)	79686	200 µl
AKT1, Inactive, His-tag	40000	100 µg
AKT2, His-tag	40011	10 µg
AKT2, Inactive, His-tag	40001	100 µg
AKT3, His-tag	40012	10 µg