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Data Sheet

GSK3α Assay Kit

Catalog #79698

Background: GSK3 (Glycogen synthase kinase) is a serine/threonine kinase which initially was found to phosphorylate and inactivate glycogen synthase in glycogen biosynthesis. It is an important regulator enzyme in many disease pathogeneses including cancer, immune disorders, metabolic disorders and neurological disorders like Alzheimer's disease. Recently, GSK3α, one of the isoforms of GSK3, was identified as a key target in acute myeloid leukemia (AML).

Description: The *GSK3α Assay Kit* is designed to measure GSK3α activity for screening and profiling applications using Kinase-Glo® (Promega) as a detection reagent. The *GSK3α Assay Kit* comes in a convenient 96-well format, with enough purified recombinant GSK3α enzyme, GSK3α substrate (GSK substrate peptide), ATP, and kinase assay buffer for 100 enzyme reactions.

COMPONENTS:

Catalog #	Reagent	Amount	Storage	
40006	GSK3α	1.5 µg	-80°C	***Avoid multiple freeze/ thaw cycles!***
79334	5x Kinase assay buffer	1.5 ml	-20°C	
79686	ATP (500 µM)	100 µl	-20°C	
79697	10x GSK substrate peptide	500 µl	-20°C	
	96-well plate, white	1	Room Temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Kinase-Glo® Max Assay (Promega #V6071)
Dithiothreitol (DTT, 1 M; optional)
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.

CONTRAINDICATION: Keep final DMSO concentration below 1%

REFERENCE:
McCubrey JA., *et al. Oncotarget* **5(10):** 2881-2911 (2014)

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

1) Thaw **5x Kinase assay buffer, ATP** and **10x GSK substrate peptide.** (Optional: If desired, add DTT to **5x Kinase assay buffer** to make a 10 mM concentration; *e.g.* add 10 µl of 1 M DTT to 1 ml **5x Kinase assay buffer.** Prepare only enough 5x Kinase assay buffer with DTT as required for the assay, as any excess 5x kinase buffer/DTT cannot be stored and should be discarded.)

2) Prepare the master mixture (25 µl per well): N wells x (5 µl **5x Kinase assay buffer** + 1 µl **ATP (500 µM)** + 5 µl **10x GSK substrate peptide** + 14 µl distilled water). Add 25 µl to every well.

	Positive Control	Test Inhibitor	Blank
5x Kinase assay buffer	5 µl	5 µl	5 µl
ATP (500 µM)	1 µl	1 µl	1 µl
10x GSK substrate peptide	5 µl	5 µl	5 µl
Water	14 µl	14 µl	14 µl
Test Inhibitor	–	5 µl	–
10% DMSO in water (Inhibitor buffer)	5 µl	–	5 µl
1x Kinase buffer	–	–	20 µl
GSK3α (~0.6 ng/µl)	20 µl	20 µl	–
Total	50 µl	50 µl	50 µl

3) 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 dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor 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 1% DMSO concentration in the final reaction mixture.*

4) Add 5 µl of Inhibitor solution to each well labeled as “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of 10% DMSO in water (Inhibitor buffer).

5) Prepare 3 ml of **1x Kinase assay buffer** by mixing 600 µl of **5x Kinase assay buffer** with 2400 µl water. (3 ml of **1x Kinase assay buffer** is sufficient for 100 reactions.) Dilute only enough **5x kinase assay buffer** as required for the assay; discard any remaining **1x kinase assay buffer.**

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

7) Thaw **GSK3α enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Calculate the amount of **GSK3α** required for the assay and dilute enzyme to ~0.6 ng/µl with **1x Kinase assay buffer**. Store remaining undiluted enzyme in aliquots at -80°C. *Note: GSK3α enzyme is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

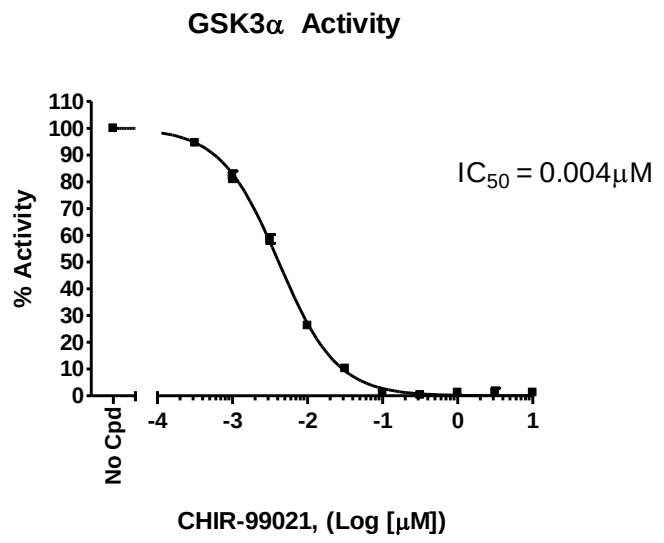
8) Initiate reaction by adding 20 µl of **diluted GSK3α enzyme** to the wells designated "Positive Control" and "Test Inhibitor Control." Carefully shake the plate well and incubate it at 30˚C for 45 minutes.

9) Thaw Kinase-Glo Max reagent.

10) 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.

11) Measure luminescence using the microplate reader. "Blank" value should be subtracted from all wells.

Example of Assay Results:



Inhibition of GSK3α enzyme by CHIR99021, measured using the *GSK3α kinase assay kit* (Cat. #79698). *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
GSK3α	40006	10 µg
GSK3β	40007	10 µg
TCF/LEF Reporter Kit (Wnt Signaling Pathway)	60500	500 rxns
CRE/CREB Reporter Kit (cAMP/PKA Signaling Pathway)	60611	500 rxns
CRE/CREB Luciferase Reporter Lentivirus	79580	500 µl x 2

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