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Data Sheet

Fluorogenic GCN5 Assay Kit

Catalog #: 50091

DESCRIPTION: The *Fluorogenic GCN5 Assay Kit* is a homogeneous, fluorogenic assay designed to screen for inhibitors of GCN5. It comes in a convenient 96-well format, with all the reagents necessary for 100 fluorescent HAT activity measurements. No time-consuming washing steps are required. In addition, the kit includes purified GCN5 (also known as KAT2A) for use as a positive control. The *Fluorogenic GCN5 Assay Kit* is based on the transfer of an acetyl group from acetyl CoA to a peptide substrate. After incubation with acetyl CoA and the substrate, the GCN5 enzyme generates acetylated H3 peptide and CoASH. The thiol groups of CoASH can be detected with fluorogenic reagent at excitation = 360 nm and emission = 460 nm.

COMPONENTS:

Catalog	Component	Amount	Storage	
50074	GCN5 (KAT2A) enzyme	25 µg	-80°C	***Avoid freeze/thaw cycles!***
	Acetyl CoA (1 mM)	100 µl	-80°C	
50095	10X HAT assay buffer	5 ml	-20°C	
52010	H3 peptide	500 µl	-20°C	
	Fluorescence Developer	250 µl	-20°C	
	Developer Solution	10 ml	-20°C	
50096	HAT Stop Solution	5 ml	Room temp.	
	black, low binding NUNC black microtiter plate	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of excitation in the range of 350-380 nm and detection in the range of 440-460 nm
Adjustable micropipettor and sterile tips

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

CONTRAINDICATIONS: DTT, β-mercaptoethanol, or other reducing reagents, DMSO >1%, strong acids or bases, ionic detergents, high salt

REFERENCE(S): Trievel, R. C., *et al.* (2000). *Anal. Biochem.* **287(2):**319-28.

STABILITY: 12 months from date of receipt when stored as directed.

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

Immediately prior to assay:
Dilute GCN5 in 1X HAT Assay buffer to 50 ng/µl (250 ng/reaction). Aliquot any remaining enzyme and store undiluted at -80°C. Keep diluted enzyme on ice. Discard any remaining diluted enzyme after use. *Note: GCN5 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

Step 1:
In duplicate, add the reaction mixtures (below) to the black microtiter plate. Be sure to add the GCN5 enzyme last so all reactions start simultaneously. Incubate at 37ºC for 30 min.

	Enzyme Positive Control	Test Inhibitor	Auto-acetylation Control	"Blank" Negative Control
H_2O	34 µl	34 - X µl	39 µl	39 µl
10X HAT assay buffer	5 µl	5 µl	5 µl	5 µl
Acetyl-CoA	1 µl	1 µl	1 µl	1 µl
H3 peptide	5 µl	5 µl	–	5 µl
Test Inhibitor	–	X µl	–	–
GCN5 (50 ng/µl)	5 µl	5 µl	5 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2: Stop the reaction
Add 50 µl of Stop Solution to each well. No incubation is required; proceed to Step 3.

Step 3: Fluorescence development
1. Dilute the Fluorescence Developer in Developer Solution (1:50). Make only sufficient quantity needed for the assay; discard any remaining diluted developer.
2. Add 100 µl diluted developer to each well.
3. Incubate at room temperature for 20 minutes.

Step 4: Fluorescence Measurement
Measure the fluorescence in a fluorescence plate reader at excitation 360 nm and emission 460 nm. "Blank" value is subtracted from all readings.

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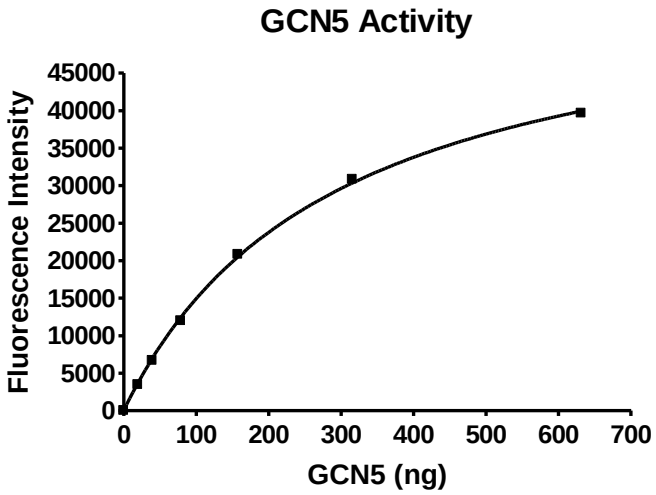
Example of Assay Results:



GCN5 enzyme activity, measured using the *Fluorogenic GCN5 Assay Kit*, Cat. #50091. Fluorescence was measured using a Bio-Tek fluorescent microplate reader.
Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS

GCN5 Enzyme	#50074	100 µg
HAT Assay Buffer	#50095	20 mL
HAT Stop Solution	#50096	20 mL
Fluorogenic P300 Assay Kit	#50092	96 rxns
P300 Enzyme	#50071	50 µg
HDAC Assay Kit	#50033	96 rxns
HDAC Class 2a Assay Kit	#50041	96 rxns
HDAC8 Assay Kit	#50068	96 rxns
HDAC1 enzyme	#50051	50 µg
HDAC2 enzyme	#50002	50 µg
HDAC3/NcoR2 enzyme	#50003	50 µg
HDAC4 enzyme	#50004	2 µg
HDAC5 enzyme	#50005	10 µg
HDAC6 enzyme	#50006	50 µg