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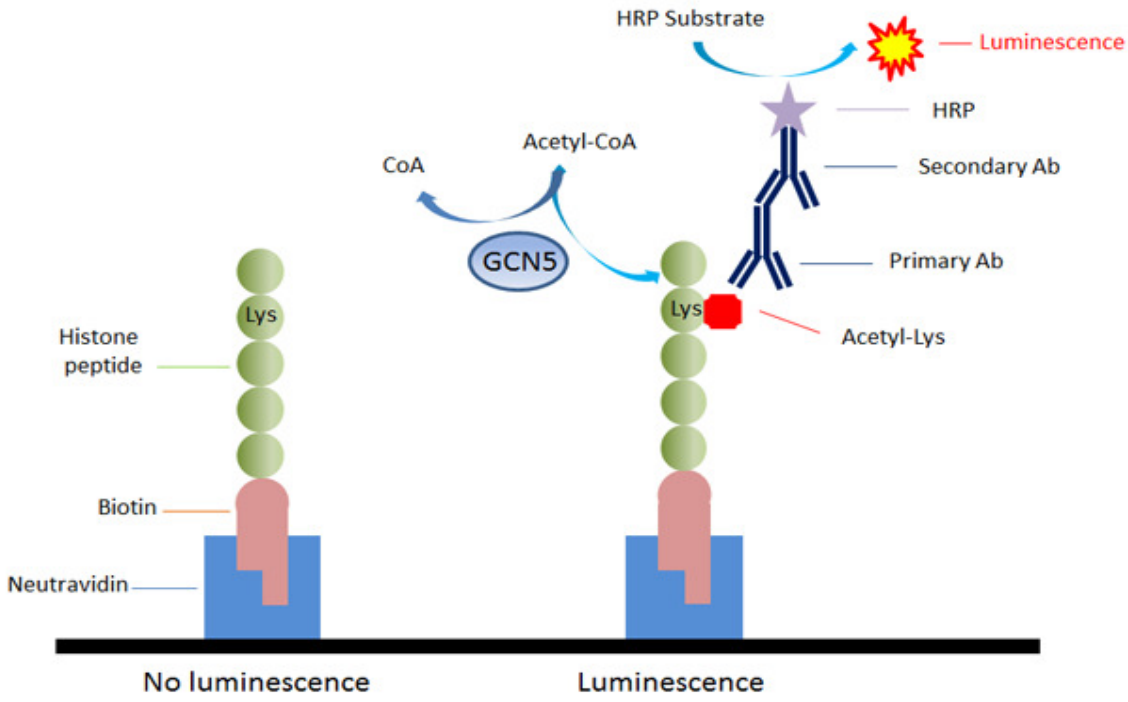
Data Sheet

GCN5 Chemiluminescent Assay Kit

Catalog #: 50079
Size: 96 reactions

DESCRIPTION: The *GCN5 Chemiluminescent Assay Kit* is an enzyme-linked immunosorbent assay (ELISA) designed to screen for inhibitors of GCN5. Histone acetyltransferase GCN5 (also known as KAT2A) is involved in various cellular events, and its dysfunction is linked to a number of human diseases including cancers and diabetes. The *GCN5 Chemiluminescent Assay Kit* comes in a convenient format, with a 96-well plate precoated with Neutravidin and Histone peptide, and all the reagents necessary for 96 chemiluminescent GCN5 activity measurements. In addition, the kit includes purified GCN5 for use as a positive control. The *GCN5 Chemiluminescent Assay Kit* is based on the GCN5 enzyme transferring an acetyl group from an acetyl donor (acetyl CoA) to a histone substrate. The acetylated histone is recognized by a highly specific primary antibody, followed by an HRP-labeled secondary antibody. The chemiluminescence produced by HRP can be measured using a chemiluminescence reader.



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COMPONENTS:

Catalog	Component	Amount	Storage	
50074	GCN5 human recombinant enzyme	20 µg	-80°C	***Avoid freeze/thaw cycles!***
	Acetyl CoA (1 mM)	500 µl	-20°C	
50095	10X HAT assay buffer	10 ml	-20°C	
52140U	Primary antibody 21	50 µl	-20°C	
52131H	Secondary antibody 2	12 µl	-20°C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
52100	Blocking buffer	50 ml	+4°C	
	White microplate (or strips) precoated with Neutravidin and Histone peptide	1 plate	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Tween-20
TBST buffer (1 x Tris-Buffered Saline, pH 8.0, containing 0.05% Tween-20)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips
Rotating or rocker platform

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

CONTRAINDICATIONS: DMSO >1%, strong acids or bases, ionic detergents, high salt.

REFERENCE(S):
1. Chen, L., *et al.* (2013). *J. Biol. Chem.* **17(20):** 14510-21.
2. Dominy, J. E. Jr., *et al.* (2010). Biochim. Biophys. Acta. **1804(8):** 1676-83.

STABILITY: 6 months from date of receipt when stored as directed.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Rehydrate the microwells by adding 200 µl of TBST buffer (1x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 5 minutes at room temperature. Remove liquid and tap the plate onto clean paper towels to remove remaining liquid.
2) Prepare the master mixture: N wells × (5 µl **10X HAT Assay Buffer** + 5 µl **Acetyl-CoA** (1 mM) + 30 µl water). Add 40 µl of master mixture to all wells labeled “Positive

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Control", "Test Inhibitor" and "Blank". To the wells labeled "Substrate Control", add 5 µl **10X HAT Assay Buffer** + 35 µl water.

3) Add 5 µl of inhibitor solution of each well designated "Test Inhibitor". For the wells labeled "Positive Control", "Substrate Control", and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer).

4) Add 5 µl of **1X HAT Assay Buffer** to the wells designated "Blank".

5) Thaw **GCN5 enzyme** on ice. Aliquot the enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: GCN5 enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **GCN5** in **1X HAT Assay Buffer** at 20 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

7) Initiate reaction by adding 5 µl of **diluted GCN5** to the wells designated "Positive Control", "Substrate Control", and "Test Sample ". Incubate at room temperature for 1 hour.

8) Remove the reaction solution and wash the wells three times with 200 µl TBST buffer and blot dry onto clean paper towels.

	Positive Control	**Test Inhibitor**	**Substrate Control**	**"Blank" Negative Control**
H_2O	30 µl	30 µl	35 µl	30 µl
10X HAT Assay Buffer	5 µl	5 µl	5 µl	5 µl
Acetyl-CoA (1 mM)	5 µl	5 µl	–	5 µl
Test Inhibitor	–	5 µl	–	–
Inhibitor buffer (no inhibitor)	5 µl	–	5 µl	5 µl
1X HAT Assay Buffer	–	–	–	5 µl
GCN5 (20 ng/µl)	5 µl	5 µl	5 µl	–
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:

1) Dilute **Primary antibody 21** 200-fold with **Blocking buffer** plus 0.05% Tween-20.

2) Add 100 µl per well. Incubate 1 hour at room temperature with slow shaking.

3) Wash the wells three times with 200 µl TBST buffer and blot dry onto clean paper towels.

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Step 3:

1) Dilute **Secondary antibody 2** 1,000-fold with **Blocking buffer** plus 0.05% Tween-20.
2) Add 100 µl per well. Incubate 30 minutes at room temperature with slow shaking.
3) Wash plate three times with 200 µl TBST buffer and blot dry onto clean paper towels.

Step 4:

1) *Just before use,* mix **HRP Chemiluminescent Substrate A** and **HRP Chemiluminescent substrate B** with 1:1 ratio and add 100 µl the mixture to each well. Discard any unused chemiluminescent reagent after use.
2) Immediately read samples in a luminometer or microtiter-plate reader capable of reading chemiluminescence. "Blank" value is subtracted from all other values.

Reading Chemiluminescence:
Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavenlength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 sec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: 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).

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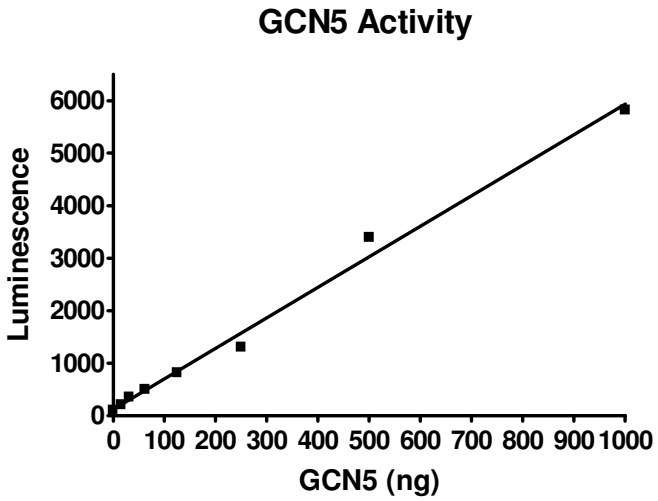
Example of Assay Results:



GCN5 enzyme activity, measured using the *GCN5 Chemiluminescent Assay Kit*, Cat. #50079. Luminescent intensity 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

Product Name	Catalog	Size
GCN5 (KAT2A) Enzyme	#50074	50 µg
P300 Enzyme	#50071	50 µg
ATAT1 Enzyme	#50072	50 µg
HAT Assay Buffer	#50095	20 ml
HAT Stop Solution	#50096	20 ml
Fluorogenic GCN5 Assay Kit	#50091	96 rxns
Fluorogenic p300 Assay Kit	#50092	96 rxns
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
HDAC8 enzyme	#50008	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	GCN5 enzyme has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh enzyme (GCN5, BPS Bioscience # 50074). Store enzyme in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection. See section on "Reading Chemiluminescence" above.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of enzyme (GCN5, BPS Bioscience # 50074) to create a standard curve.

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