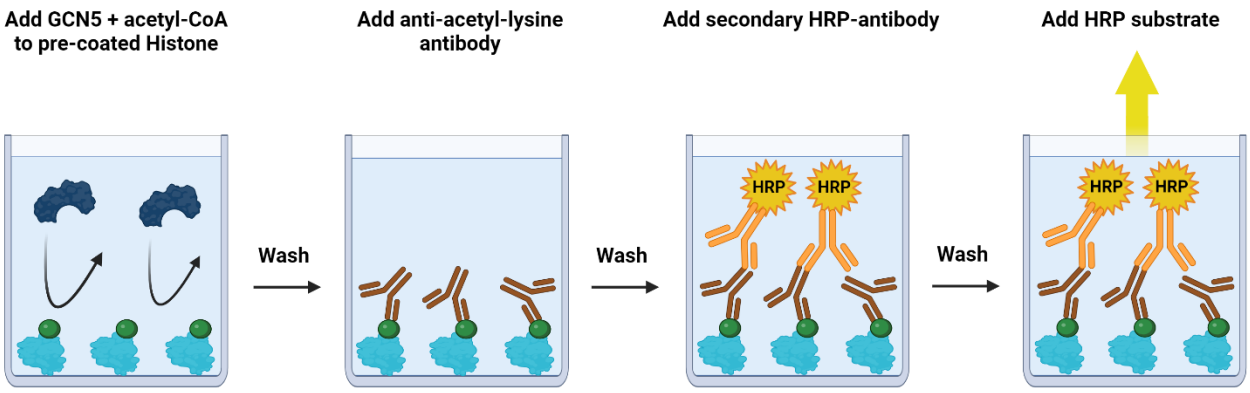
Description

The GCN5 Chemiluminescent Assay Kit is an ELISA (enzyme-linked immunosorbent assay)-based assay designed to measure the histone acetyltransferase activity of GCN5 ((General control of amino acid synthesis, Yeast, Homolog)-like 2) for screening and profiling applications. The GCN5 assay kit comes in a convenient 96-well format, with enough recombinant purified GCN5 enzyme (amino acids 362-837), acetyl donor, pre-coated plate with histone substrate, all the reagents necessary for assay detection and blocking buffer for 96 enzyme reactions.



Created with Biorender.

Figure 1: Schematic representation of the mechanism behind GCN5 Chemiluminescent Assay Kit.
GCN5 protein is added to a plate pre-coated with a histone substrate in the presence of acetyl-CoA. After washing, an anti-acetyl-lysine antibody is added, followed by a secondary HRP-antibody. Lastly, HRP substrate is added and the chemiluminescence signal generated can be measured. The signal is proportional to the acetyltransferase activity of GCN5.

Background

GCN5 ((General control of amino acid synthesis, Yeast, Homolog)-like 2), also known as KAT2A or lysine acetyltransferase 2A, can function as acetyltransferase, glutaryltransferase, succinyltransferase or malonyltransferase, depending on the cellular context, and it functions as a transcription regulator. As part of the SAGA (SPT-ADA-GCN5 acetyltransferase) and ATAC (ADA2A containing complex) complexes it functions as a histone acetyltransferase (HAT) on core histones. SAGA is a 19-subunit complex made of 4 units, including GCN5, that can recruit transcription proteins and modify promoter-proximal chromatin, being also involved in DNA damage repair and signaling pathways. ATAC can serve as a co-factor for c-Jun in the transcription of JNK (Jun N-terminal kinase) target genes. In the context of the hematopoietic lineage, GCN5 regulates maturation of NKT (natural killer T) cells, differentiation of granulocytic cells and proliferation of T cells. Both SAGA and ATAC have been linked to cancer and seem to be essential for cancer cell survival. A deep understanding of the GCN5 functions, and ability to inhibit or downregulate it may prove beneficial for patients suffering cancer where SAGA and ATAC are essential for cancer cell survival.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
50074	GCN5 (KAT2A), FLAG-Tag*	5 µg	-80°C
	0.1 mM Acetyl-CoA	10 µl	-20°C
79708	2x HAT Assay Buffer	10 ml	-20°C
52140U	Primary Antibody 21	12 µl	-20°C
52131H	Secondary HRP-Labeled Antibody 2	10 µl	-20°C
52100	Blocking Buffer 4	50 ml	+4°C
	0.5M DTT	200 µl	-20°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
	White plate (or strips) pre-coated with Histone-Substrate	1 plate	+4°C

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- TBST Buffer (1x Tris-buffered saline, pH 8, containing 0.05% Tween-20)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

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.

Contraindications

The GCN5 Chemiluminescent Assay Kit is compatible with up to 1% final DMSO concentration. Avoid the use of strong acids and bases, ionic detergents and high salt concentrations.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Inhibitor" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Butyrolactone 3 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

Step 1:

1. Rehydrate each pre-coated well with 200 µl of TBST Buffer.

2. Incubate 5 minutes at Room Temperature (RT).

3. Tap the plate onto clean paper towel to remove the liquid.

4. Wash the plate three times using 200 µl TBST Buffer per well.

5. Tap the plate onto clean paper towel to remove the liquid.

6. 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.

   6.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in TBST.

   For the positive and negative controls, use TBST Buffer (Diluent Solution).

   OR

   6.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with TBST Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in TBST Buffer to keep the concentration of DMSO constant.

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

   Note: The final concentration of DMSO should not exceed 1%.

7. Thaw **GCN5** on ice. Briefly spin the tube containing the enzyme to recover its full content.

8. Dilute GCN5 to 2 ng/µl in TBST Buffer (20 µl/well).

9. Tap the plate onto clean paper towel to remove the liquid.

10. Add 20 µl TBST Buffer to the "Blank" wells.

11. Add 5 µl of inhibitor solution to the "Test Inhibitor" wells.

12. Add 5 µl of Diluent Solution to the "Blank" and "Positive Control" wells.

13. Add 20 µl of diluted GCN5 solution to the "Positive Control" and "Test Inhibitor" wells.

14. Incubate at RT for 30 minutes.

15. Prepare the Acetyl-Coa Substrate Solution (25 µl/well). For example, to prepare 3 ml of solution mix 2982 µl of 2x HAT Assay Buffer, 12 µl of 0.5M DTT, and 6 µl of 0.1 mM Acetyl-CoA.

16. Add 25 µl of Acetyl-CoA Substrate Solution to each well.

	Blank	**Positive Control**	**Test Inhibitor**
TBST Buffer	20 µl	-	-
Diluted GCN5 (2 ng/µl)	-	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Incubate at RT for 30 minutes			
Acetyl-Coa Substrate Solution	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

17. Incubate at 30°C for one hour.

18. Tap the plate onto clean paper towel to remove the Acetyl-Coa Substrate Solution.

19. Wash the plate three times using 200 µl TBST Buffer per well.

20. Tap the plate onto clean paper towel to remove the liquid.

21. Add 100 µl of Blocking Buffer 4 into each well.

22. Incubate at RT for 10 minutes.

23. Tap the plate onto clean paper towel to remove the Blocking Solution.

24. Wash the plate three times using 200 µl TBST Buffer per well.

25. Tap the plate onto clean paper towel to remove the liquid.

Step 2:

1. Dilute 1000-fold Primary Antibody 21 with Blocking Buffer 4 (100 µl/well).
2. Add 100 µl of diluted Primary Antibody 21 to each well.
3. Incubate 1 hour at RT with gentle agitation.
4. Wash the plate three times using 200 µl TBST Buffer per well.
5. Add 100 µl of Blocking Buffer 4 into each well.
6. Incubate at RT for 10 minutes.
7. Tap the plate onto clean paper towel to remove the Blocking Solution.
8. Wash the plate three times using 200 µl TBST Buffer per well.
9. Tap the plate onto clean paper towel to remove the liquid.

Step 3:

1. Dilute 1000-fold Secondary Antibody 2 with Blocking Buffer 4 (100 µl/well).
2. Add 100 µl of diluted Secondary Antibody 2 to each well.
3. Incubate 30 minutes at RT with gentle agitation.
4. Wash the plate three times using 200 µl TBST Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 µl of Blocking Buffer 4 into each well.
7. Incubate at RT for 10 minutes.
8. Tap the plate onto clean paper towel to remove the Blocking Solution.
9. Wash the plate three times using 200 µl TBST Buffer per well.
10. Tap the plate onto clean paper towel to remove the liquid.

Step 4:

1. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

2. Add 100 µl of mix per well.

3. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

4. The "Blank" value should be 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 wavelength 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 msec. 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).

Example Results

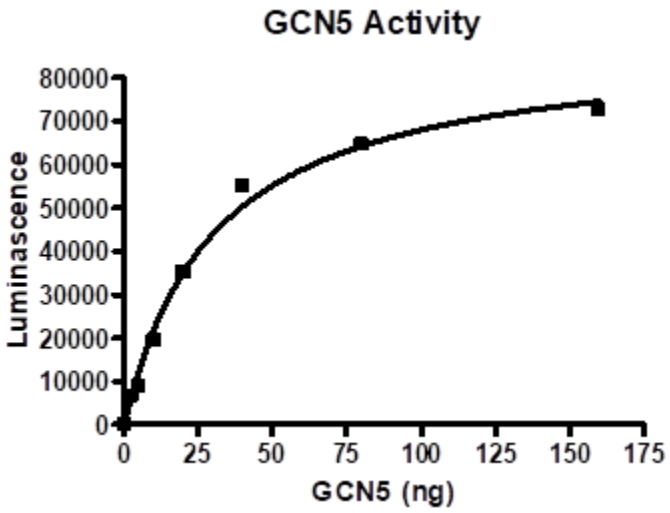


Figure 2: GCN5 enzyme activity.
GCN5 enzyme activity was measured using increasing amounts of enzyme. Luminescent intensity was measured using a Bio-Tek fluorescent microplate reader.

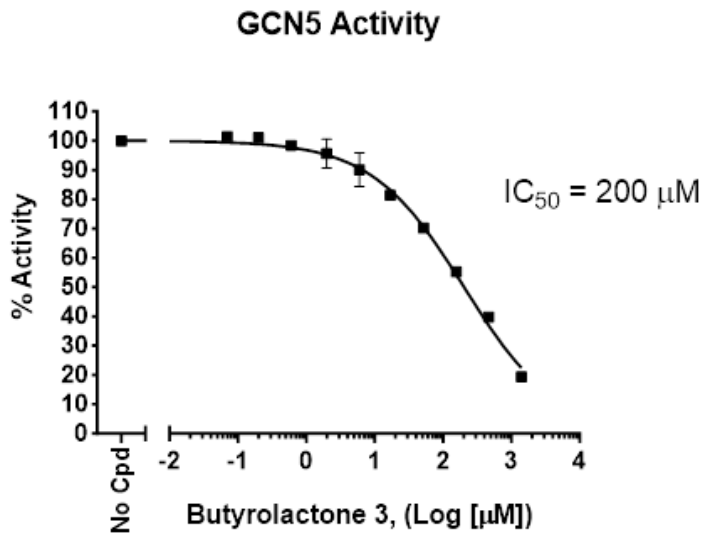


Figure 3: Inhibition of GCN5 activity by Butyrolactone 3.
GCN5 inhibition was measured in the presence of increasing concentrations of Butyrolactone 3 (MedChemExpress HY-129039). Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

References

Herbst D., *et al.*, 2021 *Nature Structural & Molecular Biology* 28: 989-996.
Suganuma T., *et al.*, 2010 *Cell* 142 (5): 726-736.
Arede L., *et al.*, 2022 *Blood Adv.* 6(1): 165-180.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
GCN5 (727-837), His-Tag Recombinant	31114	100 µg
PCAF (KAT2B), His-Tag Recombinant	31120	100 µg
Chemi-Verse™ CDK3/CyclinE1 Kinase Assay Kit	78884	96 reactions
CDK3/CyclinE1, GST-Tag Recombinant	40103	10 µg

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