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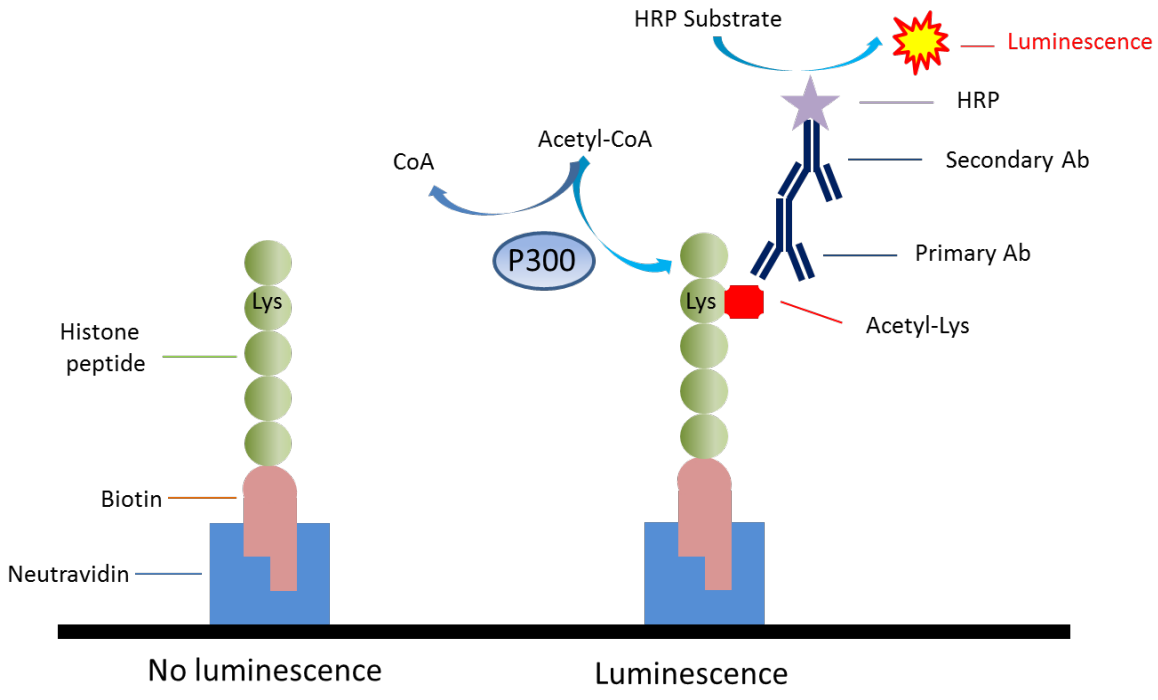
Data Sheet

P300 Chemiluminescent Assay Kit

Catalog #: 79705

DESCRIPTION: The *p300 Chemiluminescent Assay Kit* is an enzyme-linked immunosorbent assay (ELISA) designed to screen for inhibitors of p300 or to measure histone acetyltransferase (HAT) activity for screening and profiling applications. Histone acetyltransferase p300 (also known as KAT3B) is involved in various cellular events, and its dysfunction is linked to a number of human diseases including Parkinson's, Huntington's, and Alzheimer's diseases. The *p300 Chemiluminescent Assay Kit* comes in a convenient format, with Histone peptide, a 96-well plate precoated with Neutravidin and histone peptide, and all the reagents necessary for 96 chemiluminescent P300 activity measurements. In addition, the kit includes purified p300 for use as a positive control. The *P300 Chemiluminescent Assay Kit* is based on the p300 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	
50071	P300 human recombinant enzyme	>1 µg	-80°C	***Avoid freeze/thaw cycles!***
	Acetyl CoA (0.1 mM)	10 µl	-20°C	
79708	2X HAT assay buffer	10 ml	-20°C	
	0.5 M DTT	20 µl	-20°C	
79706	Primary antibody 327	10 µl	-80°C	
52131H	Secondary HRP-labeled antibody 2	10 µl	-80°C	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room Temp.	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp.	
52100	Blocking buffer 4	50 ml	+4°C	
	96-well plate precoated with histone substrate	1 plate	+4°C	

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): Ravindra, K. C., *et al.* (2012). *Chem Res Toxicol.* **25(2):** 337–347.

STABILITY: At least 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 (1 x TBS, pH 8.0, containing 0.05% Tween-20) to every well. Incubate 15 minutes at room temperature.
2) During the incubation, dilute the inhibitor to the concentration 10-fold higher than the final testing concentration in TBST.

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3) Thaw **P300 human recombinant enzyme** on ice. Aliquot the enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: **P300 human recombinant enzyme** enzyme is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute **P300 human recombinant enzyme** in TBST Buffer at 0.01 ng/µl.

5) Remove TBST buffer and tap the plate onto clean paper towels to remove remaining liquid.

6) Add 20 µl TBST buffer to Blank or Negative control well(s).

7) Add 5 µl the inhibitor solution to the inhibitor testing wells. Add 5 µl of 10% DMSO in water (Inhibitor buffer) to Blank and Positive control wells. *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 into the 50 µl assay to make a 1% DMSO concentration in the final reaction mixture.*

8) Add 20 µl of P300 solution (0.01 ng/µl) to Positive control and inhibitor testing wells. Incubate the plate at room temperature for 30 minutes.

9) Incubate the plate at room temperature for 30 minutes.

10) Prepare the Acetyl-CoA substrate solution: N wells × 25 µl substrate solution, prepared as follows:

    For 100 wells, prepare 3000 µl Acetyl-CoA solution (always prepare a little more) by mixing 2982 µl of 2X HAT assay buffer, 12 µl of 0.5 M DTT and 6 µl of 0.1 mM Acetyl-CoA solution. For smaller numbers of wells, scale the volumes appropriately.

11) Add 25 µl of Acetyl-CoA substrate solution to each well. Incubate the reaction at 30°C for one hour.

12) Remove supernatant from the wells and wash the strip three times with 200 µl of TBST buffer. Blot dry onto paper towels.

13) Add 100 µl of **Blocking Buffer 4** into each well and incubate at room temperature for 10 minutes.

14) Remove supernatant from the wells and wash the strip three times with 200 µl of TBST buffer. Blot dry onto paper towels.

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	"Blank" Negative Control	Positive Control	Test Inhibitor
TBST	20 µl	–	–
P300 (0.01 ng/µl)	–	20 µl	20 µl
10% DMSO in water (Inhibitor buffer)	5 µl	5 µl	–
Test Inhibitor	–	–	5 µl
Incubate at room temperature for 30 minutes			
Substrate solution (Step 11)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**
Incubate at 30°C for one hour			

Step 2:

1) Dilute **Primary antibody 327** 1000-fold with **Blocking buffer 4**.
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.
4) Add 100 µl of **Blocking Buffer 4** into each well and incubate at room temperature for 10 minutes.
5) Wash the wells three times with 200 µl of TBST and blot dry onto paper towels.

Step 3:

1) Dilute **Secondary HRP-labeled antibody 2** 1,000-fold with **Blocking buffer 4**.
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.
4) Add 100 µl of **Blocking Buffer 4** into each well and incubate at room temperature for 10 minutes.
5) Wash plate three times with 200 µl TBST buffer and blot dry onto paper towels.

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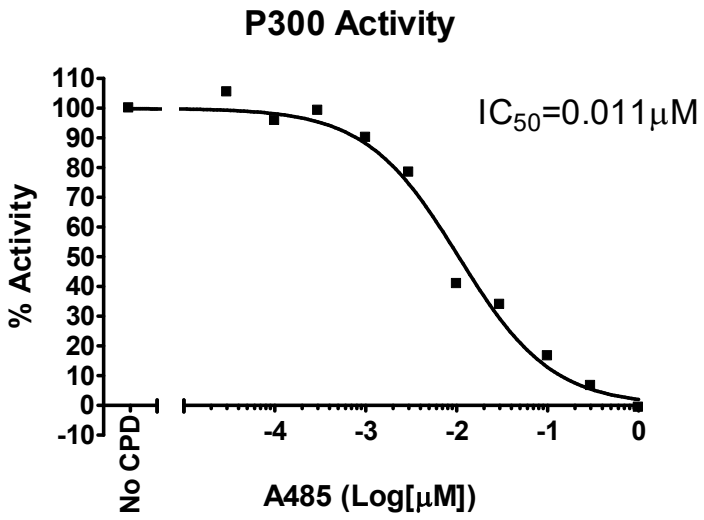
Step 4:

1) *Just before use,* mix **ELISA ECL Substrate A** and **ELISA ECL 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 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 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).

Example of Assay Results:



Inhibition of P300 enzyme activity by A485, measured using the *Chemiluminescent P300 Assay Kit*, Cat. #79705. 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*

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RELATED PRODUCTS

Product Name	Catalog	Size
P300 Enzyme	#50071	50 µg
GCN5 Enzyme	#50074	50 µg
ATAT1 Enzyme	#50072	50 µg
10X HAT Assay Buffer	#50095	20 ml
HAT Stop Solution	#50096	20 ml
GCN5 Chemiluminescent Assay Kit	#50079L	96 rxns

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