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Data Sheet

Fluorogenic P300 Assay Kit

Catalog #: 50092

DESCRIPTION: The *Fluorogenic p300 Assay Kit* is a homogeneous, fluorogenic assay designed to screen for inhibitors of p300. 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 p300 (also known as KAT3B) for use as a positive control. The *Fluorogenic p300 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 P300 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	Avoid freeze/thaw cycles!
50071	P300 human recombinant enzyme	10 µg	-80 °C	
	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 P300 in 1X HAT Assay buffer to 20 ng/μl (100 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: P300 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 P300 enzyme last so all reactions start simultaneously. Incubate at 37°C for 30 min.

	Enzyme Positive Control	Test Inhibitor	Auto-acetylation Control	"Blank" Negative Control
H ₂ O	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	–	–
P300 (20 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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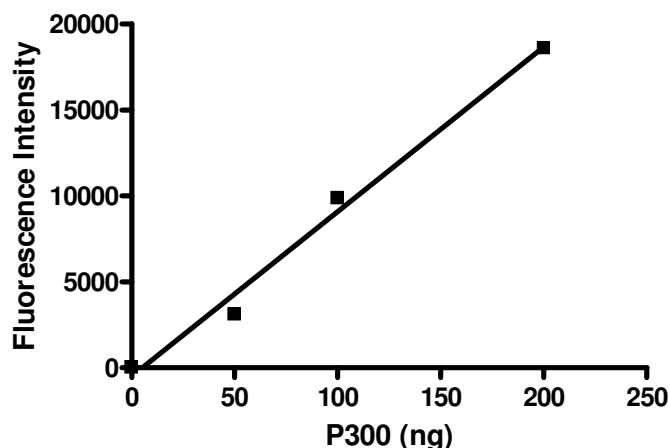
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Example of Assay Results:



P300 enzyme activity, measured using the *Fluorogenic P300 Assay Kit*, Cat. #50092. 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

<u>Product Name</u>	<u>Catalog</u>	<u>Size</u>
P300 Enzyme	#50071	50 µg
HAT Assay Buffer	#50095	20 mL
HAT Stop Solution	#50096	20 mL
Fluorogenic GCN5 Assay Kit	#50091	96 rxns
GCN5 Enzyme	#50070	100 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

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