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Data Sheet

P300 Homogeneous Assay Kit

Catalog #: w60089
Size: 384 reactions

DESCRIPTION: The *p300 Homogeneous Assay Kit* contains an AlphaLISA® assay designed for screening inhibitors of p300 in a convenient 384-well format. This efficient method requires no time-consuming washing steps. Only a few simple steps on a microtiter plate are needed. The assay is based on the enzymatic transfer of an acetyl group from acetyl CoA to a specific lysine residue within a biotinylated peptide substrate. After the p300 reaction, the sample is incubated with a combination of an antibody that specifically binds the acetylated peptide and acceptor beads that bind the antibody. This is followed by incubation with streptavidin-labeled donor beads and reading of the Alpha-counts.

COMPONENTS:

Catalog	Component	Amount	Storage	
w60082	P300 human recombinant enzyme	1 µg	-80 ℃	***Avoid freeze/thaw cycles!***
	Acetyl CoA (1 mM)	500 µl	-80 ℃	
	3X HAT assay buffer	4 ml	-80 ℃	
	Biotinylated H3 Peptide Substrate	700 µl	-80 ℃	
	Primary Antibody 20	40 µl	-20 ℃	
	4X Detection Buffer	2 ml	-80 ℃	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Anti-Rabbit AlphaLISA® Acceptor Beads, 5 mg/ml (PerkinElmer #AL104)
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002)
Optiplate-384 (PerkinElmer #6007290)
AlphaScreen® microplate reader
Adjustable micropipettor and sterile tips

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

CONTRAINDICATIONS: DMSO concentrations above 0.5%. Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen® assays.

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

WEST BIOSCIENCE

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

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

1) Prepare the master mixture: N wells × (2.5 µl 3× **HAT Assay Buffer** + 1.5 µl **Biotinylated H3 Peptide Substrate** + 1 µl **Acetyl-CoA**).

2) Add 5 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 2.5 µl **3× HAT Assay Buffer** + 1.5 µl H_2O +1 µl of **Acetyl-CoA**.

3) Thaw **P300 enzyme** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Aliquot protein into single use aliquots. Store remaining undiluted protein in aliquots at -80℃ immediately. *Note: **P300** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

4) Dilute **P300** in **1x HAT Assay Buffer** to 0.25 ng/µl (0.6 ng/reaction). Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Positive Control	**Test Inhibitor**	**Substrate Control**	**"Blank" Negative Control**
3X HAT Assay Buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Biotinylated H3 Peptide Substrate	1.5 µl	1.5 µl	–	1.5 µl
Acetyl-CoA	1 µl	1 µl	1 µl	1 µl
Water	–	–	1.5 µl	–
Test Inhibitor	–	2.5 µl	–	–
Inhibitor buffer (no inhibitor)	2.5 µl	–	2.5 µl	2.5 µl
1 X HAT Assay Buffer	–	–	–	2.5 µl
P300 (0.25 ng/µl)	2.5 µl	2.5 µl	2.5 µL	–
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

5) Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2.5 µl of the same solution without inhibitor (inhibitor buffer). *Note: Keep DMSO concentration below 0.5 %.*

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6) Add 2.5 µl of **1x HAT Assay Buffer** to the well designated "Blank".
7) Initiate reaction by adding 2.5 µl of diluted P300 prepared as described above to the wells labeled "Positive Control", "Substrate Control", and "Test Inhibitor". Incubate at 37°C for 30 minutes.

Step 2:

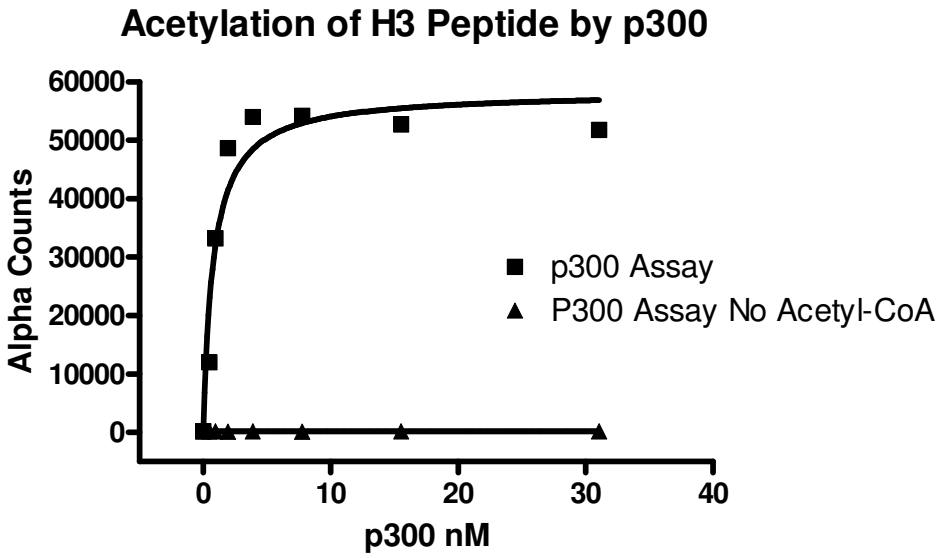
Note: Protect your samples from direct exposure to light!

1) In a single solution, dilute anti-Rabbit AlphaLISA Acceptor Beads (PerkinElmer #AL104) (1:500) and **Primary Antibody 20** (1:100) with **1x Detection Buffer**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002) 125-fold with **1x Detection Buffer**. Add 10 µl per well. Incubate at room temperature for 30 minutes.
2) Read Alpha-counts.

Example of Assay Results:



p300 enzyme activity, measured using the p300 Assay Kit, West Bioscience, Catalog # w60089. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*

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