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Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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 steps 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 incubation with p300, 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.

Background

Histone acetyltransferase p300, also known as p300 HAT or EP300, is a histone acetyltransferase that regulates gene transcription through chromatin remodeling. Therefore, p300 plays an important role in the regulation of cell proliferation or differentiation. p300 is closely related to CREB binding protein. It interacts with many transcription factors. It also mediates cAMP-gene regulation by binding specifically to the phosphorylated CREB protein. Mutations in p300 are involved in the rare genetic disease Rubinstein–Taybi syndrome and in several types of cancer, including stomach, colon, breast, and pancreatic cancer.

Applications

- Study enzyme kinetics of p300
- Screen small molecular inhibitors for drug discovery and High throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
50071	Human recombinant P300 enzyme*	1 µg	-80°C
	Acetyl CoA (0.1 mM)	10 µl	-20°C
	Biotinylated H3 Peptide Substrate	400 µl	-80°C
79708	2X HAT assay buffer	10 ml	-20°C
	0.5 M DTT	200 µl	-20°C
79706	Primary Antibody 327	40 µl	-80°C
52301	4X Detection Buffer 1	2 ml	-80°C

**The concentration of p300 is lot-specific and will be indicated on the tube containing the enzyme.*

Materials Required but Not Supplied

Name	Catalog #
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	

BPS Bioscience
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Storage Conditions

This assay kit will perform optimally for up to 12 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.

Assay Principle

AlphaLISA immunoassays are a no-wash alternative to ELISA immunoassays using the proprietary system developed by PerkinElmer. These assays are robust and, being homogeneous, require a minimal hands-on approach. The Streptavadin-coated Alpha donor bead is bound to a biotinylated anti-analyte, while the AlphaLISA acceptor bead is conjugated with an anti-analyte. With the presence of an analyte, the beads move closely to one another and upon excitation, initiate an Alpha signal that changes the emission wavelength which is then measured.

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.

Assay Protocol

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

STEP 1:

1. Add 0.5 M DTT to **2x HAT assay buffer**, to bring the final concentration of DTT to 2 mM. For example, add 4 µl of 0.5 M DTT to 996 µl of **2x HAT assay buffer** . Do not freeze and re-use the thawed assay buffer once DTT has been added.

2. Dilute **Acetyl-CoA** to 50 nM in distilled water. Prepare only enough Acetyl-CoA as needed for the assay. Do not freeze and re-use the diluted Acetyl-CoA.

3. Prepare the Master Mix: N wells × (3 µl of **2x HAT Assay Buffer** (containing DTT) + 1 µl of Biotinylated H3 Peptide Substrate + 2 µl of 50 nM Acetyl-CoA)

4. Add 6 µl of Master Mix to each well designated “Positive Control”, “Test Inhibitor”, and “Blank”.

5. Prepare 1x HAT Assay Buffer by adding 1 part **2x HAT Assay Buffer** (containing DTT) to 1 part distilled water.

6. Prepare serial dilutions of the Test Inhibitor using 1x **HAT Assay Buffer.**
7. Add 2 µl of Test Inhibitor to each well designated "Test Inhibitor". For the "Positive Control" and "Blank", add 2 µl of the diluent solution without inhibitor . The diluent solution should contain the same concentration of solvent as the test inhibitor (for example DMSO in 1x HAT assay buffer if the inhibitor was dissolved in DMSO).

   Note: Keep the final DMSO concentration below 0.5%.
8. Add 2 µl of **1x HAT Assay Buffer** to the well designated "Blank".
9. Thaw **P300 enzyme** on ice. Briefly spin the tube containing the protein to recover the full content of the tube. ***Note: p300 is very sensitive to freeze/thaw cycles. Do not re-use the diluted protein.***
10. Dilute **p300** in 1x HAT Assay Buffer to 0.5 ng/µl (1 ng/reaction). Keep the diluted protein on ice until use. Discard any unused diluted protein after use.
11. Initiate the reaction by adding 2 µl of diluted **p300** to the wells labeled "Positive Control", and "Test Inhibitor".

    Incubate at 37°C for 30 minutes.

Component	Blank	Positive Control	Test Inhibitor
Master Mix	6 µl	6 µl	6 µl
Test Inhibitor	-	-	2 µl
Diluent solution	2 µl	2 µl	-
1 X HAT Assay Buffer	2 µl	-	-
P300 (0.5 ng/µl)	-	2 µl	2 µl
Total	**10 µl**	**10 µl**	**10 µl**

STEP 2:

Protect your samples from direct exposure to light. Photobleaching will occur.

1. Prepare 1x **Detection Buffer** by adding 1 part **4x Detection Buffer** to 3 parts distilled water.
2. In a single solution, dilute the anti-Rabbit AlphaLISA® Acceptor Beads (PerkinElmer #AL104) (500-fold) and **Primary Antibody 327** (100 fold) with **1x Detection Buffer**. Add 10 µl per well to all wells. Shake the plate briefly. Incubate at room temperature for 30 minutes.

STEP 3:

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

Example Results

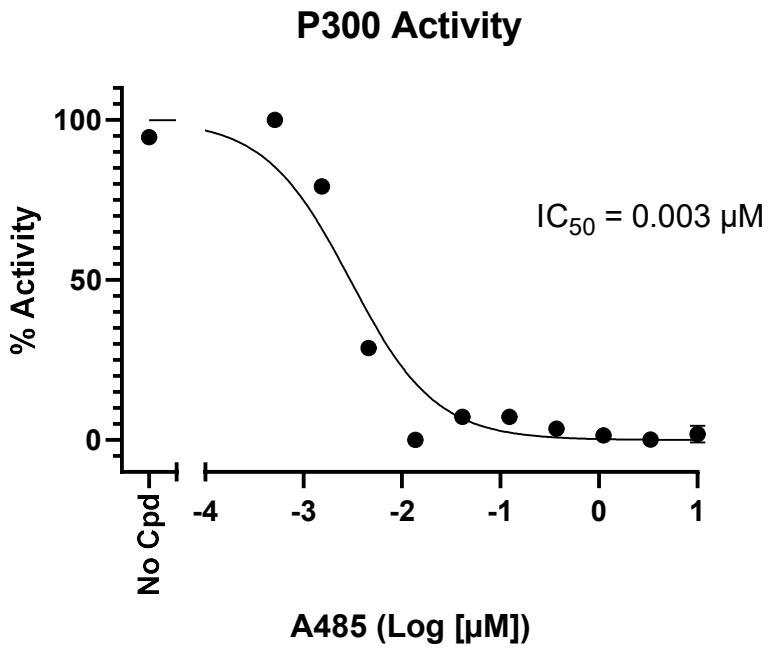

Figure 1: Inhibition of p300 enzymatic activity by A-485. p300 enzyme activity was measured using the p300 Assay Kit (BPS Bioscience, #50078) in the presence of increasing concentration of the acetyltransferase inhibitor A-485. Blank values were subtracted from all other values. Results are expressed as percent of "positive control" (set at 100% and measured in the absence of inhibitor).

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

General Considerations

Plates and Instruments: A plate reader capable of Alpha technology detection is required. We recommend using PerkinElmer 384-Optiplate #6007290.

"Blank" Control: The "Blank" control is important to determine the background signal in the assay. We recommend doing it in duplicate.

Troubleshooting Guide

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

References

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

Related Products

Products	*Catalog #*	*Size*
p300 Enzyme	50071	50 µg
p300 (1046-1163), His-tag	31118	100 µg
HAT Assay Buffer	50095	20 mL
HAT Stop Solution	50096	20 mL
p300 Chemiluminescent Assay Kit	79705	96 rxns
GCN5 Enzyme	50070	100 µg
ATAT1 Enzyme	50072	50 µg