MA-0146

p300 Inhibitor Screening Assay

(100 wells, Fluorometric, Ex/Em = 372/465 nm, Store at -80°C)

Introduction:

p300, also known as KAT3B, is a histone acetyltransferase. One of the primary functions of p300 is its role in epigenetic regulation. By acetylating histones, p300 alters the structure of chromatin, promoting transcriptional activation and accessibility of genes. This process allows the cells to respond dynamically to external cues and ensure appropriate gene expression during development, tissue homeostasis, and cellular response to stress and environmental changes. p300 interacts with a wide range of transcription factors and co-regulators, acting as a bridge between signaling pathways and gene expression. Its diverse interactions enable p300 to mediate complex cellular processes, such as cell cycle progression, differentiation, and apoptosis. Dysregulation of p300 activity has been implicated in various diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases, making it an attractive target for therapeutic interventions. AkrivisBio's p300 Inhibitor Screening Assay is a simple method of assessing potential p300 inhibitors in a rapid and high-throughput adaptable assay.

Assay Principle:

1 – P300 in the well utilizes acetyl CoA to acetylate a model substrate (H3 peptide- aa 1-21), forming Coenzyme A.
2 – The free thiol group of coenzyme A is recognized by 7-Diethylamino-3-(4'-Maleimidylphenyl)-4-Methylcoumarin (CPM), a thiol specific reagent, resulting in a large increase in fluorescence proportional to the amount of p300 activity.
3 – In the presence of an inhibitor, the rate of fluorescence increase is greatly attenuated.

Screening Assay Components:

Assay Buffer	20 ml	WM	MA-0146A
p300 Enzyme	100 µl	Green	MA-0146B
Acetyl CoA	Lyoph	Red	MA-0146C
H3 Peptide	Lyoph	Amber	MA-0146D
CPM Reagent	200 µl	Violet	MA-0146E
Anacardic Acid	10 µl	Blue	MA-0146F

User Supplied Reagents:

- Dimethyl sulfoxide
- Isopropanol

Storage and Handling Considerations:

Store the kit at -80°C. When first opened, centrifuge vials briefly before opening. It is recommended to thaw and aliquot the p300 enzyme, Acetyl CoA, H3 peptide and CPM to convenient portions and store at -80°C to avoid repeated freeze/thaw cycles.
Assay Buffer: Warm to room temperature before use. Once thawed, store at 4°C.
p300 Enzyme: Store at -80°C. Keep on ice while in use.
Acetyl CoA: Reconstitute with 220 µl DI H_2O. Keep on ice while in use. Store at -80°C.
H3 Peptide: Reconstitute with 330 µl Assay Buffer. Keep on ice while in use. Store at -80°C.
CPM Reagent: Thaw just before using**.** Make sure DMSO is completely melted. Store at -20°C
Anacardic Acid: 5 mM solution in DMSO. Ready to use as supplied. Store at -20°C.

Screening Protocol:

1. Control Wells: Proper use of this screening assay involves the use of several different control wells, namely a Background Control (omits substrate, Coenzyme A) to correct for fluorescence not due to p300 activity, Enzyme Control (omits inhibitor) to show fluorescence of uninhibited enzyme activity, Solvent Control (includes solvent but not inhibitor) to correct any apparent inhibition caused by the solvent used for the inhibitor and Inhibitor Control (includes a well known inhibitor) to show effective inhibition.

2. Inhibitor Test Wells: Place a container of isopropyl alcohol into a freezer at -20°C for use later in the protocol. Dissolve test inhibitors into an appropriate solvent at the highest possible inhibitor concentration (preferably 10X or higher than highest final inhibitor concentration). Dilute the inhibitors to 4x the highest desired test concentration with Assay Buffer. Prepare Diluted Solvent only at the same ratio. Add 25 µl of diluted test inhibitors to wells of a 96-well plate. Any planned serial dilution of the inhibitors is done now. Bring all wells containing inhibitors to 25 µl with Assay Buffer.

3. Prepare the Control wells:

	Background Control	Enzyme Control	Solvent Control	Inhibitor Control	(Test Wells)
Assay Buffer	25 µl	25 µl	------	24 µl	------
Diluted Solvent only	------	------	25 µl	-----	------
Anacardic Acid	------	------	------	1 µl	------

4. p300 Enzyme: All wells will require 25 µl of p300 enzyme solution. Prepare sufficient enzyme solution for the number of wells to be run, containing:

Assay Buffer	22 µl
p300 Enzyme	1 µl
Acetyl CoA	2 µl

Add 25 µl to all wells to be run. Mix and incubate at 30°C for 10 minutes to allow inhibitor binding to occur.

5. H3 Peptide Substrate: All wells EXCEPT the Background Control well will require 25 µl of H3 Peptide Substrate solution. Prepare sufficient H3 Peptide Substrate solution for the number of wells to be run, containing:

Assay Buffer	22 µl
H3 Peptide	3 µl

Add 25 µl of Assay Buffer to the Background Control well. Add 25 µl of H3 Peptide Substrate solution to all the other wells. Place on a plate shaker at 30°C for 30 minutes to allow the reaction to proceed. The volume of each well is 75 µl at this stage.

6. Stop the reaction: Add 50 µl of pre-chilled isopropyl alcohol into each well and mix thoroughly.

7. Detection: Prepare the CPM thiol reagent immediately before use. Each well to be analyzed will require 50 µl of CPM solution, containing:

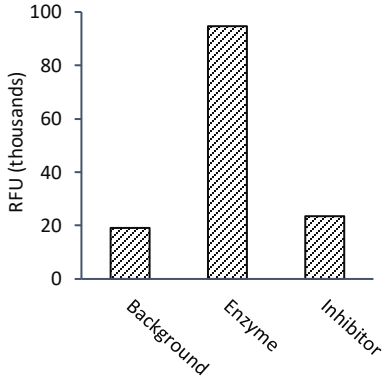
DMSO	48 µl
CPM	2 µl

Add 50 µl of CPM Thiol Reagent to each well, mix and incubate at room temperature for 15 min.

8. Measurement: Measure the fluorescence in endpoint mode, using Excitation at 372 nm, Emission at 465 nm.

9. Typical Results:

	RFU	% Inhibition
Background control	19111	100
Inhibitor control	23505	94.2
Enzyme control	94686	0



10. Calculations: Subtract the Background Control reading from all other readings. This will serve as Zero Enzyme Activity (100% Inhibition). Set the Enzyme Control as 100% Enzyme Activity (0% Inhibition). Anacardic Acid in the Inhibitor Control is present at about 70 µM, which should give an inhibition of ≥ 90%.

Residual Enzyme Activity = (Inhibitor Reading - Background Control) / (Enzyme Control – Background Control)

% Inhibition = (1 – Residual Enzyme Activity) X 100

For the Anacardic Acid Inhibitor Control in this example: Residual Enzyme Activity = (2372 – 1676) / 15047 – 1676) = 0.0521

% Inhibition = (1 - 0.0521) X 100 = 94.78

Note:

In the event that there is noticeable solvent inhibition, use the Solvent Control value as the Background Control.

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