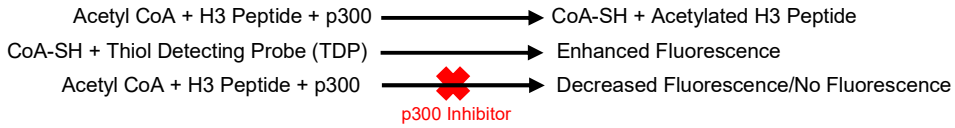
p300 Inhibitor Screening Kit (Fluorometric)

(Catalog # BN00610; 100 assays; Store kit at -80°C)

I. Introduction:

p300 (EC 2.3.1.32) or KAT3B, a histone acetyltransferase, contributes to transcriptional activation by acetylating chromatin on the lysine residues of H3 and H4 histones. There is growing evidence that p300 plays an important role in cancer cell proliferation and differentiation. p300 inhibitors have potential applications in cancer therapy. Assay Genie's p300 Inhibitor Screening Kit utilizes a H3 peptide and Acetyl CoA as the substrates. p300 acetylates the peptide and generates CoA-SH with a free thiol group. The CoA is detected using a Thiol Detecting Probe that reacts with thiol groups and gives enhanced fluorescence that can be measured at Ex/Em = 392/482 nm. In the presence of p300 specific inhibitors, the enzymatic activity is inhibited resulting in decreased or total loss of fluorescence. This assay kit is a simple, sensitive, and rapid tool to screen potential inhibitors of p300.



II. Application:

- Screening/studying/characterizing potential inhibitors of p300

III. Kit Contents:

Components	BN00610	Cap Code	Part Number
p300 Assay Buffer	20 ml	WM	BN00610-1
p300 Enzyme	0.1 ml	Green	BN00610-2
Acetyl CoA (Lyophilized)	1 vial	Red	BN00610-3
H3 Peptide (Lyophilized)	1 vial	Brown	BN00610-4
Thiol Detecting Probe	0.2 ml	Violet	BN00610-5
p300 Inhibitor (Anacardic Acid, 5 mM)	10 µl	Blue	BN00610-6

IV. User Supplied Reagents and Equipment:

- 96-well plate with flat bottom. White plates are preferred for this assay.
- Multi-well spectrophotometer (ELISA reader)
- Dimethylsulfoxide (DMSO)
- Isopropyl Alcohol pre-chilled at -20°C

V. Storage Conditions and Reagent Preparation:

Store kit at -80°C, protected from light. After thawing, briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- **p300 Assay Buffer:** Warm to room temperature before use. Once thawed, store at 4°C.
- **p300 Enzyme:** Store at -80°C. Once thawed, aliquot and store at -80°C. Keep on ice while in use. Use within two months.
- **Acetyl CoA:** Reconstitute with 220 µl dH_2O just before use and aliquot and store at -80ºC. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **H3 Peptide:** Reconstitute with 330 µl p300 Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- **Thiol Detecting Probe:** Store at -20°C or -80°C. Thaw and mix well before use.
- **p300 Inhibitor (Anacardic Acid):** Store at -20°C or -80°C. Use within two months once thawed.

VI. p300 Inhibitor Screening Protocol:

1. **Screening Compounds, Inhibitor Control & Blank Control Preparation:** Dissolve test inhibitors into proper solvent to make stock solution. Dilute to 4x the desired test concentration with p300 Assay Buffer. Add 25 µl diluted test inhibitor (Sample, S) or 25 µl of p300 Assay Buffer (Enzyme Control [EC] and *Blank Control [BC]) into desired wells. For the Inhibitor Control, add 1 µl Anacardic Acid and 24 µl p300 Assay Buffer into Inhibitor Control (IC) well(s). If desired, serial dilutions of test inhibitors may be performed at this time, to a final volume of 25 µl.

   Note:

   a) Final solvent concentration should not be more than 2%. If solvent exceeds 2%, include a Solvent Control to test the effect of the solvent on enzyme activity.

   (b) * Thiol Detecting Probe reacts with the thiol groups in the enzyme p300 and CoA. Hence a Blank Control (BC) containing p300 and CoA should be used.

2. **p300 Enzyme Solution Preparation:** Prepare 25 µl of p300 enzyme solution for each well containing test inhibitors, Enzyme Control, Blank Control, and Inhibitor Control.

p300 Assay Buffer	24 µl
p300 Enzyme	1 µl

Mix and add 25 µl/well to the designated wells. Mix well. Incubate at 30°C for 10 min.

3. **p300 Substrate Preparation:** For S, EC and IC, prepare 50 µl/well of p300 substrate solution. For blank control well(s), prepare 50 µl/well of BC solution.

	p300 Substrate Solution	BC Solution
p300 Assay Buffer	45 µl	48 µl
Acetyl CoA	2 µl	2 µl
H3 Peptide	3 µl	----

Mix & add 50 µl of p300 Substrate Solution and BC Solution into desired well(s). Mix well. Incubate at 30°C for 30 min. Stop the reaction by adding 50 µl of pre-chilled isopropyl alcohol (not provided) into each well and mix. For each well, prepare 50 µl of Thiol Detecting Probe working solution by adding 2 µl Thiol Detecting Probe into 48 µl of DMSO (not provided) just before use. Add 50 µl of Thiol Detecting Probe working solution into each well, mix and incubate at room temperature for 15 min.

4. **Measurement**: Measure fluorescence (Ex/Em = 392/482 nm).
5. **Calculations:** Subtract the BC RFU value from RFU values of all reactions. Calculate the % Inhibition as shown below.

$$\%\ \textbf{Inhibition} = \frac{\textbf{RFU of EC} - \textbf{RFU of S}}{\textbf{RFU of EC}} \times \textbf{100}$$

Notes:

a) If RFU of Solvent Control is less than RFU of Enzyme Control, then make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent, or use a different solvent.

b) If RFU of test inhibitor is lower than RFU for BC, treat it as 100 % inhibition and further dilute the test inhibitor and repeat the assay.

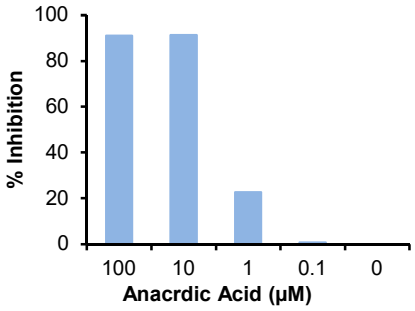


Figure: Inhibition of p300 activity by the p300 Inhibitor [Anacardic Acid] (Cat. # 1849). Assays were performed following the kit protocol.

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