

pCAF Inhibitor Screening Kit (Fluorometric)

(Catalog BN00609; 100 assays; Store at -20°C)

I. Introduction:

pCAF (P300/CBP-associated factor, EC 2.3.1.48) also known as K (lysine) acetyltransferase 2B (KAT2B), possesses intrinsic histone acetyltransferase (HAT) activity. pCAF contributes to transcriptional activation by acetylating chromatin and transcription factors through its HAT activity. Deregulated HAT activity plays an important role in various cancers. pCAF inhibitors have potential applications in cancer therapy. pCAF uses both histone 3 (H3) and histone 4 (H4) as substrates. Assay Genie's pCAF Inhibitor Screening Kit utilizes H3 peptide and Acetyl CoA as the substrates. pCAF acetylates the peptide and generates Coenzyme A 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 pCAF 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 pCAF inhibitors.



II. Application:

- Screening/studying/characterizing potential inhibitors of pCAF

III. Kit Contents:

Components	BN00609	Cap Code	Part Number
pCAF Assay Buffer	20 ml	WM	BN00609-1
pCAF Enzyme	40 µl	Green	BN00609-2
Acetyl CoA (Lyophilized)	1 vial	Red	BN00609-3
H3 Peptide (Lyophilized)	1 vial	Brown	BN00609-4
Thiol Detecting Probe	200 µl	Violet	BN00609-5
pCAF Inhibitor (Garcinol, 10 mM)	10 µl	Blue	BN00609-6

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 prechilled at -20°C.

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. After thawing, briefly centrifuge small vials at low speed prior to opening. Read the entire protocol before performing the experiment.

- pCAF Assay Buffer:** Warm to room temperature before use. Store at 4°C or -20°C.
- pCAF Enzyme:** Aliquot and store at -20°C. Keep on ice while in use. Use within two months. Don't vortex the enzyme. Gently mix by pipetting up & down.
- Acetyl CoA:** Reconstitute with 220 µl pCAF Assay Buffer just before use, aliquot and store at -80°C. Avoid repeated freeze/thaw. Keep on ice while in use. Use within two months.
- H3 Peptide:** Reconstitute with 330 µl pCAF 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. Thaw and mix well before use.
- pCAF Inhibitor (Garcinol):** Store at -20°C. Protect from air & light. Use within two months once thawed.

VI. pCAF 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 pCAF Assay Buffer. Add 25 µl diluted test inhibitor (Sample, S) or 25 µl of pCAF Assay Buffer (Enzyme Control [EC] and *Blank Control [BC]) into desired wells. For the Inhibitor Control, add 1 µl Garcinol Inhibitor and 24 µl pCAF 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.

Notes:

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

2. pCAF Enzyme Solution Preparation: Dilute pCAF Enzyme 1:10 in pCAF Assay Buffer just before use. For each well containing test inhibitors, Enzyme Control, Blank Control, and Inhibitor Control, prepare 25 µl of pCAF enzyme working solution.

pCAF Assay Buffer	21 µl
Diluted pCAF Enzyme	4 µl

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

Note: Diluted pCAF Enzyme can be stored at -80°C. Use diluted enzyme within a week.

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

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

Mix & add 50 µl of pCAF Substrate Solution and BC Solution into the desired wells. Mix well. Incubate at 30°C for 10 min. Stop the reaction by adding 50 µl of prechilled isopropyl alcohol (not provided) into each well. For each well, prepare 50 µl of Thiol Detection Probe working solution by adding 2 µl of 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 well, 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.

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

Notes:

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

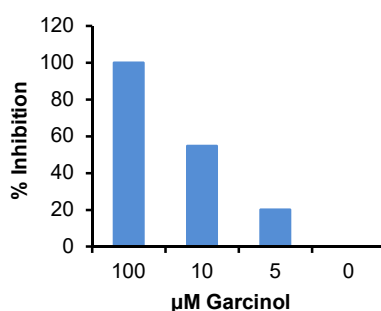


Figure: Inhibition of pCAF activity by the pCAF Inhibitor [Garcinol]. Assays were performed following the kit protocol.

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