ab196996
KAT3B/p300 Inhibitor Screening Assay Kit (Fluorometric)

Instructions for Use

For the rapid, sensitive and accurate screening of KAT3B/p300 inhibitors.

This product is for research use only and is not intended for diagnostic use.

Version 2 Last Updated 4 February 2019

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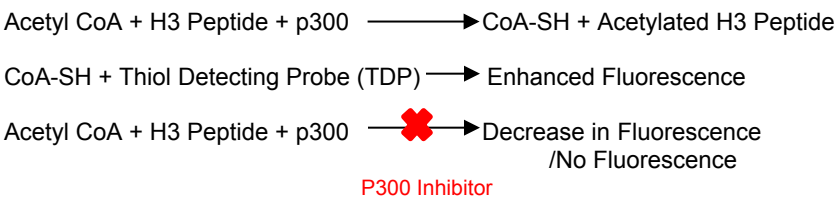
1. BACKGROUND

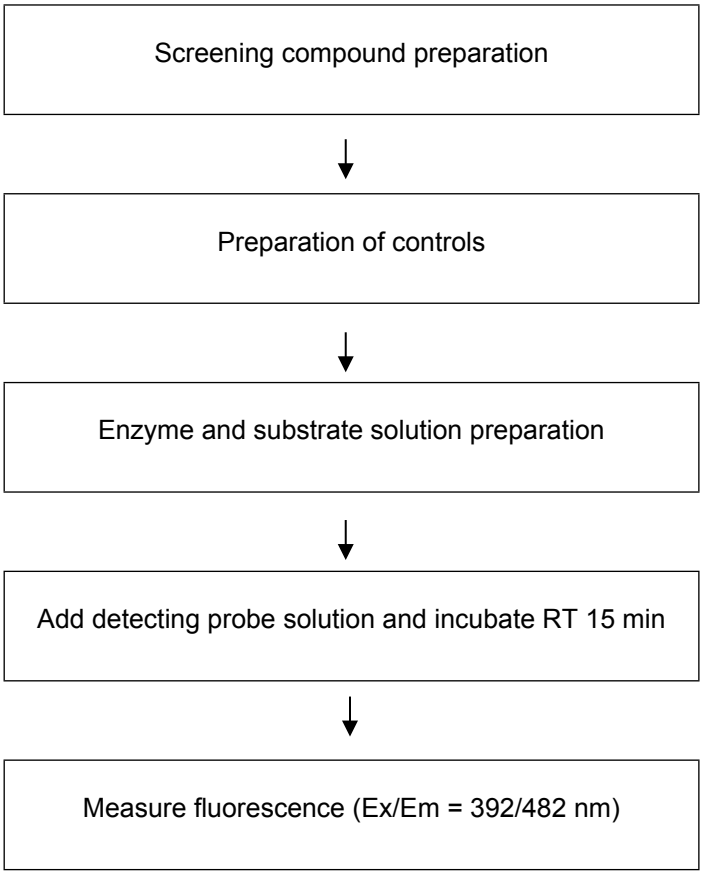
KAT3B/p300 Inhibitor Screening Assay Kit (Fluorometric) (ab196996) is an assay where histone H3 peptide and Acetyl CoA are used as acetylation substrates. KAT3B/p300 acetylates the Histone H3 peptide and generates Coenzyme A with a free thiol group (CoA-SH). CoA-SH reacts subsequently with a Thiol Detecting Probe in order to increase the amount of fluorescence in the solution, which can be measured at Ex/Em = 392/482 nm. In the presence of KAT2B/p300 specific inhibitors, the enzymatic activity is reduced ot completely abolished resulting in decreased or total loss of fluorescence.

This assay kit is a simple, sensitive and rapid tool to screen potential p300 inhibitors.

KTA3B (K-(lysine) acetyltransferase 3B, EC 2.3.1.32), also known as p300, possesses intrinsic histone acetyltransferase (HAT) activity and is able to acetylate lysine residues present in both histone H3 and histone H4. There is growing evidence that p300 plays an important role in cancer cell proliferation and differentiation. p300 inhibitors have potential applications in cancer therapy.



2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -80ºC in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
p300 Assay Buffer	20 mL	-80°C (Dry Ice)	4°C
p300 Enzyme	100 µL	-80°C (Dry Ice)	-80°C
Acetyl CoA (Lyophilized)	1 vial	-80°C (Dry Ice)	-80°C
H3 Peptide (Lyophilized)	1 vial	-80°C (Dry Ice)	-80°C
Thiol Detecting Probe	200 µL	-80°C (Dry Ice)	-20°CC
p300 Inhibitor (Anacardic acid, 5 mM)	10 µL	-80°C (Dry Ice)	-20°C / -80°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully perform this assay:

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter for Ex/Em = 392/482 nm
- 96 well plate: black plates (clear bottoms) for fluorometric assay
- Heat block or water bath
- DMSO
- Isopropyl alcohol - prechilled at -20°C

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **p300 Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Once thawed, store at 4°C.

9.2 **p300 Enzyme:**

Ready to use as supplied. Aliquot enzyme so that you have enough volume to perform the desired number of tests. Store at -80°C. Use within 2 months. Keep on ice while in use.

9.3 **Acetyl CoA:**

Reconstitute in 220 µL of p300 Assay Buffer just before use. Aliquot acetyl CoA so that you have enough volume to perform the desired number of assays. Store at -80°C. Avoid repeated freeze/thaw. Use within 2 months. Keep on ice while in use.

9.4 **H3 Peptide:**

Reconstitute in 330 µL of p300 Assay Buffer. Pipette up and down to dissolve completely. Aliquot peptide so that you have enough volume to perform the desired number of tests. Store at -80°C. Avoid repeated freeze/thaw. Use within 2 months. Keep on ice while in use.

9.5 **Thiol Detecting Probe:**

Ready to use as supplied. Equilibrate to room temperature before use. Mix well before use. Aliquot probe so that you have enough volume to perform the desired number of tests. Store at -20°C or -80°C protected from light. Once the probe is thawed, use within two months.

9.6 **p300 Inhibitor (Anacardic Acid):**

Ready to use as supplied. Aliquot inhibitor so that you have enough volume to perform the desired number of tests. Store at -20°C or -80°C. Use with 2 months. Keep on ice while in use.

10. SAMPLE PREPARATION

- Always prepare a fresh set of samples and controls for every use.

10.1 Dissolve test inhibitors into proper solvent to make stock solution.

10.2 Dilute to 4X the desired test concentration with p300 Assay Buffer before use.

NOTE: *We suggest using different volumes of testing compounds if effective concentration is unknown.*

11. ASSAY PROCEDURE and DETECTION

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**
- **Serial dilution of test inhibitors may be performed to a final volume of 25 µL.**

11.1 Dilute p300 Enzyme (Section 9.2) 1:10 in p300 Buffer prior to use. ***NOTE:*** *Diluted p300 Enzyme can be stored at -80°C. Use diluted enzyme within 1 week.*

11.2 **Prepare p300 Enzyme Solution:**

Prepare 25 µL of p300 Enzyme Solution:

Component	Enzyme Solution (µL)
P300 Assay Buffer	**21**
Diluted p300 Enzyme (1/10)	**4**

Mix sufficient reagents for the number of assays to be performed. Prepare a master mix of the Enzyme Mix to ensure consistency.

11.3 **Set up reaction wells:**

- Sample wells (S) = 1- 25 µL test inhibitors (adjust volume to 25 µL/well with p300 Assay Buffer).
- Inhibitor Control wells (IC) = 1 µL AnarardicAcid +p300 Assay Buffer.
- Enzyme Control wells (EC) = 25 µL p300 Assay Buffer.
- Blank Control wells (BC) = 25 µL p300 Assay Buffer. ***NOTE:*** *Thiol Detecting probe reacts with the thiol groups in the enzyme and CoA.*
- OPTIONAL: Solvent control (SC) = 20 µL solvent. ***NOTE:*** *preferred final solvent concentration should not be more than 2% by volume. If solvent exceeds 2%, include solvent control to test the effect on the solvent on enzyme activity.*

The table below shows the set up reaction wells:

Component	Sample Well (S) (µL)	Blank Control (BC) (µL)	Enzyme control (EC) (µL)	Inhibitor Control (IC) (µL)
Test inhibitor compound	**25**			**0**
p300 Assay Buffer		**25**	**25**	**24**
Inhibitor				**1**

11.4 Add 25 µL of p300 Enzyme Solution (Section 11.2) to each well.

11.5 Incubate at 30°C for 10 minutes.

11.6 **p300 Substrate Solution Mix:**

Prepare 50 µL of Substrate Solution for each reaction:

Component	Substrate Solution (µL)	Blank Control (BC) Solution (µL)
p300 Assay Buffer	**45**	**48**
Acetyl CoA	**2**	**2**
H3 Peptide	**3**	**-**

Mix enough reagents for the number of assays (samples, standards and positive control) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + controls + 1).

11.7 Add 50 µL of p300 Substrate Solution to each of S, EC, IC and SC (if using) wells.

11.8 Add 50 µL of Blank Control Solution to each BC well.

11.9 Incubate at 30°C for 30 minutes.

11.10 Stop the reaction by adding 50 µL of pre-chilled isopropyl alcohol into each well.

11.11 **Thiol Detection Probe:**

Prepare 50 µL of Thiol Detection Probe working solution for wells:

Component	Reaction Mix (µL)
DMSO	**48**
Thiol Detecting Probe	**2**

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X µL component x (Number samples + controls +1).

11.1 Add 50 µL Thiol Detection Probe into each well, mix, and incubate at room temperature for 15 minutes.

11.2 Measure fluorescence in a microplate reader at Ex/Em = 392/482 nm.

12. CALCULATIONS

- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

12.1 Average the duplicate reading for each test sample compound, Inhibitor Control and Enzyme control.

12.2 Subtract the mean absorbance value of the Blank Control (BC) from all controls and sample readings. This is the corrected absorbance.

12.3 Calculate the % inhibitions as follows:

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

NOTE:

If RFU of SC < RFU of EC = make a higher stock of test inhibitor, or dissolve the inhibitor in lower concentration of the solvent; or use a different solvent.

If RFU of S < RFU of BC = treat as 100% inhibition and further dilute the test inhibitor and repeat the assay.

13. TYPICAL DATA

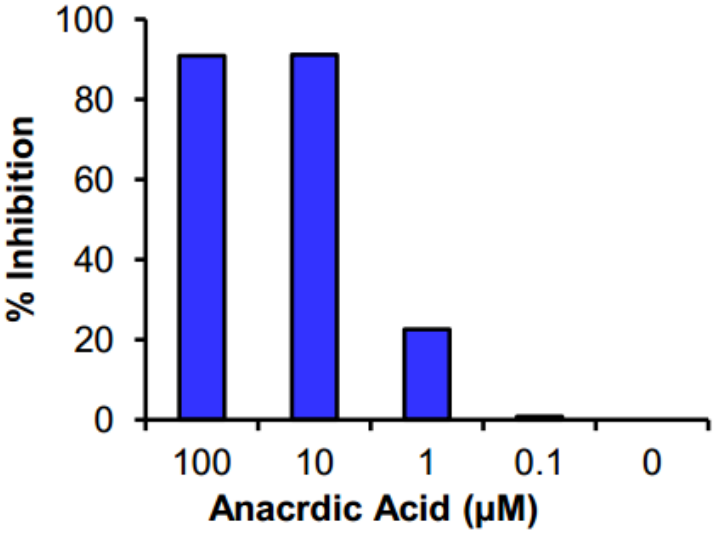


Figure 1. Inhibition of p300 activity by the p300 inhibitor (anacardic acid).

14. QUICK ASSAY PROCEDURE

***NOTE**: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.*

- Prepare standard, enzyme mix, substrate mix and Thio Detecting Probe (aliquot if necessary); get equipment ready.
- Prepare 25 µL of p300 Enzyme Working Solution for each well:

Component	Enzyme Solution (µL)
p300 Assay Buffer	**21**
Diluted p300 Enzyme (1/10)	**4**

- Set up Reaction wells as follows:

Component	Sample Well (S) (µL)	Blank Control (BC) (µL)	Enzyme control (EC) (µL)	Inhibitor Control (IC) (µL)	Solvent Control (SC) (µL)
Test inhibitor compound	**25**			**0**	
Solvent inhibitor					**25**
p300 Assay Buffer		**25**	**25**	**24**	
Inhibitor				**1**	
Add 25 µL/well of p300 Enzyme Working Solution					
Incubate at 30°C for 10 min					

- For S, EC and IC wells, prepare 50 µL of pCAF Substrate Solution. For BC wells, prepare 50 µL of Blank Control Solution:

Component	Substrate Solution (µL)	Blank Control Solution (µL)
p300 Assay Buffer	**45**	**48**
Acetyl CoA	**2**	**2**
H3 Peptide	**3**	**0**

- Add 50 µL of p300 Substrate Solution and Blank Control Solution into the appropriate wells. Mix well.
- Incubate at 30°C 30 min.

- Add 50 µL of pre-chilled isopropyl alcohol into each well.
- Prepare 50 µL of Thiol Detection Probe working solution:

Component	Reaction Mix (µL)
DMSO	**48**
Thiol Detecting Probe	**2**

- Add 50 µL of Thiol Detecting Probe working solution into each well.
- Mix well and incubate RT 15 mins.
- Measure plate at Ex/Em= 392/482 nm for fluorometric assay.

15. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

16. **FAQ**

17. **INTERFERENCES**

18. **NOTES**

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