Acetyl-CoA Fluorometric Assay Kit

Instructions for Use

For the measurement of acetyl-CoA dependent acetyltransferase activity in purified in vitro samples that do not contain reductants for many species.

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

1. BACKGROUND

Acetyl-CoA Fluorometric Assay Kit is a homogeneous mix-and-read fluorescent assay for the determination of any acetyl-CoA dependent acetyltransferase activity. It is suitable for end-point or kinetic read options, which is ideal for determining mechanism of action, kinetics, and screening candidate compounds. The assay is amendable to HTS and miniaturization. For use with purified in vitro samples. This assay is a complete kit for the screening of candidate compounds that may alter normal acetyltransferase activity.

Acetylation is an important covalent molecular modification. Acetyltransferases are enzymes that covalently transfer an acetyl group from a donor molecule (Acetyl CoA) to an acceptor. Acetyl CoA serves as a universal donor while the acceptor varies with the acetyl transferase. Acceptors include histones, kinases, transcription factors, receptors, neurotransmitter precursors like choline and serotonin, and anti-microbial agents like chloramphenicol and fluoroquinones. Acetylation can signal an increase or decrease inactivity based on the context of the message. Frequently located at critical junctions in metabolic pathways, acetyltransferases and their regulation have become attractive therapeutic targets to treat everything from insomnia to cancer.

2. ASSAY SUMMARY

ACTIVITY ASSAY:

Enzyme and reaction mix spiked with acceptor substrate added to wells

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Incubate plate for 30 minutes at Room Temperature

↓

Add isopropyl alcohol and detection solution. Incubate for 10 minutes at Room Temperature

↓

Measure fluorescence (Ex/Em = 380/520 nm) for end point / Kinetic assay

INHIBITION ASSAY:

Inhibitor compound prepared and added to wells

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Enzyme and reaction mix spiked with acceptor substrate added to wells

↓

Incubate plate for 30 minutes at Room Temperature

↓

Add isopropyl alcohol and detection solution. Incubate for 10 minutes at Room Temperature

↓

Measure fluorescence (Ex/Em = 380/520 nm) for end point / Kinetic assay

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 -20ºC in the dark immediately upon receipt, except for acetyltransferase positive control which should be stored at -80ºC. 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 the Materials Supplied section. Aliquot components in working volumes before storing at the recommended temperature.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Black Microtiter Plate	1 unit	-20ºC	-20ºC
Transferase Assay Buffer Concentrate	15 mL	-20ºC	-20ºC
Acetyltransferase Reaction Buffer Concentrate	100 µL	-20ºC	-20ºC
Transferase Detection Solution Concentrate	150 µL	-20ºC	-20ºC
Acetyltransferase Positive Control	200 µL	-80ºC	-80ºC
Foil Plate Sealer	3 units	-20ºC	-20ºC

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).
- Acetyl-CoA dependent acetyltransferase.
- Appropriate acceptor substrate.
- Inhibitor/activator compounds to be screened.
- Pipettes and pipette tips.
- Microplate shaker.
- Disposable microtubes, 0.5 and 1.5 mL.
- Microplate reader capable of reading at Ex/Em = 380/520 nm.
- Isopropyl alcohol (ice cold).

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, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Add the reagents to the side of the well to avoid contamination.
- Ensure plates are properly sealed or covered during incubation steps.

• Make sure you have the right type of plate for your detection method of choice.
• Make sure the heat block/water bath and microplate reader are switched on.
• Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
• Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results.

9. REAGENT PREPARATION

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

9.1 **Blank Microtiter Plate:** Ready to use as supplied. Store at +2-8°C.

9.2 **Foil Plate Sealer:** Ready to use as supplied. Store at +2-8°C.

9.3 **Transferase Assay Buffer Concentrate (10X):**

Prepare 1X Assay Buffer by diluting 15 mL of the supplied Transferase Assay Buffer Concentrate with 135 mL of ddH2O. Store at +2-8°C for 3 months or the kit's expiration date, whichever is earlier.

9.4 **Acetyltransferase Positive Control:**

Ready to use as supplied. Thaw positive control slowly on ice. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -80°C.

Avoid freeze/thaw cycles.

Diluted positive control: dilute 1:100 in 1X Transferase Assay Buffer. For example, for 500 µL = 5 µL positive control + 495 µL 1X Transferase Assay Buffer.

Working dilution positive control = add 5 µL of 1:100 positive control to 195 µL 1X Transferase Assay Buffer.

Diluted positive control should be kept on ice and used within 2 hours of preparation. Any unused working dilution of positive control should be discarded.

9.5 **Transfer Detection Solution Concentrate (100X):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot detection solution concentrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

9.6 **Acetyltransferase Reaction Buffer Concentrate (50X):**

Ready to use as supplied. Equilibrate to room temperature before use. Aliquot reaction buffer concentrate so that you have enough volume to perform the desired number of assays. Store at -20°C.

10.SAMPLE PREPARATION

General Sample information:

• We recommend performing several dilutions of your sample to ensure the readings are within the standard value range. Initial recommendations = 100 nM.

10.1 Purified Proteins:

This assay is suitable for use with all Acetyl CoA-dependent acetyltransferases. It is necessary to titrate each enzyme / substrate system in the assay to determine optimal conditions.

This assay should only be used to screen purified *in vitro* samples in buffer systems without reductants. Dilute the acetyltransferase of interest in the assay buffer The positive control included in the kit should be used to verify the activity of the kit components. It should not be used to calculate the concentration of acetyltransferase activity in samples.

10.2 Inhibitory compounds:

Dilute inhibitory compounds in the appropriate solvent.

Colored compounds: the positive control provided in this kit can be used to test colored compounds for interference in the assay. Before screening, colored compounds should be tittered in the assay to ensure there is no interference with the component. Prepare the acetyltransferase of interest as described above. It is recommended that an end-point assay is performed to determine the optimal concentration of enzyme/substrate to use prior to screening candidate compounds. Make serial dilutions of the acetyltransferase of interest in the assay buffer. Initial concentrations of 100 nM are recommended. A kinetic assay format is also an available option.

11.ASSAY PROCEDURE: END POINT ASSAY

• **Equilibrate all materials and prepared reagents to room temperature prior to use.**

• **It is recommended to assay all controls and samples in duplicate.**

11.1 **Prepare 1X Detection Solution:**

Prepare 1X Detection Solution by combining the appropriate reagent volumes calculated according to the formula below:

(A) Total volume required (Tv)

[Total number of wells + 6] x 100 µL

(B) Volume of Transferase Detection Solution (100X)

[Total volume of wells (Tv from A)] x 0.01

(C) Volume of 1X Transferase Assay Buffer required

[Total volume of wells (Tv from A)] x 0.99

For example, to prepare 2 mL = 20 µL of Transferase Detection concentrate (100X) + 1980 µL of 1X Transferase Assay Buffer.

1X Detection Solution should be kept on ice and used within 4 hours of preparation. Any unused working dilution of Detection Solution should be discarded.

11.2 **Prepare Acetyltransferase Reaction Mix:**

Prepare 1X Acetyltransferase Reaction Mix by combining the appropriate reagent volumes calculated according to the formula below:

(A) Total volume required (Tv)

[Total number of wells + 4] x 25 µL

(B) Volume of Acetyltransferase Reaction Buffer (50X)

[Total volume of wells (Tv from A)] x 0.02

(C) Volume of acceptor substrate required

[Determined empirically based on enzyme used] (µL)

(D) Volume of 1X Transferase Assay Buffer required

[Total volume of wells (Tv from A)] x 0.98 – [Volume of substrate required]

Prepare 1X reaction mix on ice by combining the appropriate reagent volumes calculated in B, C and D above. For example, to prepare 2 mL of Reaction mix spiked with 22µL acceptor substrate, combine the following volumes: 40µL Acetyltransferase Reaction buffer concentrate, 22 µL of acceptor substrate and 1938 µL 1X Transferase assay buffer. Diluted reaction mix should be kept on ice and used within 8 hours of preparation. Any unused 1X reaction mix should be discarded.

Set up Reaction wells:

- Test Sample wells = 25 µL sample.
- Blank wells= 25 µL 1 X Transferase Assay Buffer.
- Positive control = 50 µL 1X positive control (Section 9.4).
- Blank Positive control = 50 µL 1 X Transferase Assay Buffer.

11.4 Add 25 µL of 1X Reaction Mix to blank and Test Sample wells only.

11.5 Cover plate with foil plate sealer.

11.6 Incubate for 30 minutes at room temperature.

11.7 Pipet 50 µL of ice cold isopropyl alcohol into all wells.

11.8 Pipette 100 µL of 1X Detection Solution into all wells.

11.9 Cover plate with foil plate sealer. Incubate for 10 minutes at room temperature, without shaking.

NOTE: *Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.*

11.10 Measure output on a fluorescent microplate reader at Ex/Em = 380/520nm.

12.ASSAY PROCEDURE: KINETIC ASSAY

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

12.1 **Prepare 1X Detection Solution:**

Prepare 1X Detection Solution by combining the appropriate reagent volumes calculated according to the formula below:

(A) Total volume required (Tv)

[Total number of wells + 6] x 100 µL

(B) Volume of Transferase Detection Solution (100X)

[Total volume of wells (Tv from A)] x 0.01

(C) Volume of 1X Transferase Assay Buffer required

[Total volume of wells (Tv from A)] x 0.99

For example, to prepare 2 mL = 20 µL of Transferase Detection concentrate (100X) + 1980 µL of 1X Transferase Assay Buffer.

1X Detection Solution should be kept on ice and used within 4 hours of preparation. Any unused working dilution of Detection Solution should be discarded.

12.2 **Prepare Acetyltransferase Reaction Mix:**

Prepare 1X Acetyltransferase Reaction Mix by combining the appropriate reagent volumes calculated according to the formula below:

(A) Total volume required (Tv)

[Total number of wells + 4] x 25 µL

(B) Volume of Acetyltransferase Reaction Buffer (50X)

[Total volume of wells (Tv from A)] x 0.02

(C) Volume of acceptor substrate required

(Determined empirically based on enzyme used) (µL)

(D) Volume of 1X Transferase Assay Buffer required

[Total volume of wells (Tv from A)] x 0.98 – [volume of substrate required]

Prepare 1X reaction mix on ice by combining the appropriate reagent volumes calculated in F, G and H above. For example, to prepare 2 mL of Reaction mix spiked with 22µL acceptor substrate, combine the following volumes: 40µL Acetyltransferase Reaction buffer concentrate, 22 µL of acceptor substrate and 1938 µL 1X Transferase assay buffer. Diluted reaction mix should be kept on ice and used within 8 hours of preparation.

12.3 **Set up Reaction wells:**

- Test Sample wells = 25 µL sample.
- Blank wells= 25 µL 1 X Transferase Assay Buffer.

- Positive control = 50 µL 1X positive control (Section 9.4).
- Blank Positive control = 50 µL 1 X Transferase Assay Buffer.

12.4 Add 25 µL of 1X Reaction Mix to blank and Test Sample wells only.

12.5 Cover plate with foil plate sealer.

12.6 Incubate for the desired periods of time. Stop the reaction at each time point by adding 50 µL of ice cold isopropyl alcohol into all wells.

12.7 Pipette 100 µL of 1X Detection Solution into all wells.

12.8 Cover plate with foil plate sealer. Incubate for 10 minutes at room temperature, without shaking.

NOTE: *Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.*

12.9 Measure output on a fluorescent microplate reader at Ex/Em = 380/520nm.

13.ASSAY PROCEDURE: INHIBITION ASSAY

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**

13.1 **Prepare 1X Detection Solution:**

Prepare 1X Detection Solution by combining the appropriate reagent volumes calculated according to the formula below:

(D) Total volume required (Tv)

[Total number of wells + 6] x 100 µL

(E) Volume of Transferase Detection Solution (100X)

[Total volume of wells (Tv from A)] x 0.01

(F) Volume of 1X Transferase Assay Buffer required

[Total volume of wells (Tv from A)] x 0.99

For example, to prepare 2 mL = 20 µL of Transferase Detection concentrate (100X) + 1980 µL of 1X Transferase Assay Buffer.

1X Detection Solution should be kept on ice and used within 4 hours of preparation. Any unused working dilution of Detection Solution should be discarded.

13.2 **Prepare Acetyltransferase Reaction Mix:**

Prepare 1X Acetyltransferase Reaction Mix by combining the appropriate reagent volumes calculated according to the formula below:

(E) Total volume required (Tv)

[Total number of wells + 4] x 25 µL

(F) Volume of Acetyltransferase Reaction Buffer (50X)

[Total volume of wells (Tv from A)] x 0.02

(G) Volume of acceptor substrate required (Determined empirically based on enzyme used) (µL)

(H) Volume of 1X Transferase Assay Buffer required

[Total volume of wells (Tv from A)] x 0.98 – [volume of substrate required]

Prepare 1X reaction mix on ice by combining the appropriate reagent volumes calculated in J, K and L above. For example, to prepare 2 mL of Reaction mix spiked with 22µL acceptor substrate, combine the following volumes: 40µL Acetyltransferase Reaction buffer concentrate, 22 µL of acceptor substrate and 1938 µL 1X Transferase assay buffer. Diluted reaction mix should be kept on ice and used within 8 hours of preparation.

13.3 **Set up Reaction wells:**

- Inhibition Sample wells = 10 µL Inhibitor dilutions.
- Zero wells = 10 µL 1 X Transferase Assay Buffer.
- Blank wells= 25 µL 1 X Transferase Assay Buffer.

13.4 Pipet 15 µL of acetyltransferase sample at chosen working concentration into Inhibition Sample wells and zero wells.

13.5 Cover plate with foil seal. Incubate for 10 minutes at room temperature without shaking.

13.6 Pipet 25 µL of 1X Reaction Mix into blank, Inhibition Sample and zero wells.

13.7 Cover plate with foil plate seal. Incubate for 30 minutes with shaking* at room temperature.

13.8 Pipet 50 µL of ice cold isopropyl alcohol into all wells.

13.9 Pipet 100 µL of 1X Detection Solution into all wells.

13.10 Cover plate with foil plate sealer. Incubate for 10 minutes at room temperature, without shaking.

NOTE: *Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.*

13.11 Measure output on a fluorescent microplate reader at Ex/Em = 380/520nm.

14.ASSAY PROCEDURE: ENZYME INTERFERENCE

- **Equilibrate all materials and prepared reagents to room temperature prior to use.**
- **It is recommended to assay all controls and samples in duplicate.**
- **Prepare dilutions of the colored compound to be screened in 1X Transferase Assay Buffer.**

14.1 Follow Procedure described in Section 11 until Step 11.2.

14.2 **Set up Reaction wells:**

- Test Compound wells = 25 µL colored compound.
- Zero wells = 25 µL 1 X Transferase Assay Buffer
- Blank wells= 50 µL 1 X Transferase Assay Buffer.

14.3 Add 25 µL of 1X Reaction Mix to Blank and Test Compound wells only.

14.4 Cover plate with foil plate sealer.

14.5 Incubate for 30 minutes at room temperature.

14.6 Pipet 50 µL of ice cold isopropyl alcohol into all wells.

14.7 Pipette 100 µL of 1X Detection Solution into all wells.

14.8 Cover plate with foil plate sealer. Incubate for 10 minutes at room temperature, without shaking.

NOTE: *Shaking is preferably carried out on a suitable plate or orbital shaker set at a speed to ensure adequate mixing of the contents of the wells. The optimal speed for each shaker will vary and may range from 120-700 rpm.*

14.9 Measure output on a fluorescent microplate reader at Ex/Em = 380/520nm.

To determine whether or not colored compounds will interfere with the assay, calculate and compare the signal to noise ratio of the colored compound dilutions to the signal to noise ratio of the zero wells.

15.CALCULATIONS

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

• Several options are available for the calculation of the inhibition of acetyltransferase. We recommend that the data be handled by a software package utilizing a suitable curve fitting program to determine the percent inhibition.

If data reduction software is not readily available, the data can be calculated as follows:

For end point calculation:

15.1 Subtract the mean absorbance value of the blank from all sample readings.

15.2 Plot the mean of each duplicate RFU versus enzyme concentration.

15.3 Calculate signal to noise ratio

$$\frac{\text{Mean RFU for enzyme dilution}}{\text{Mean RFU blank}}$$

For kinetic calculation:

15.4 Plot relative fluorescence against the stop time interval.

For inhibition calculation:

15.5 Percent inhibition should be calculated for each inhibitor dilution using this formula:

$$\frac{(Mean\ Net\ Zero(non-\ inhibited\ enzyme)RFU-\ Mean\ Net\ Inhibited\ enzyme\ RFU)}{Mean\ Net\ Zero\ (non-\ inhibited\ enzyme)RFU}$$

16.TYPICAL DATA

TYPICAL ENDPOINT ASSAY RESULTS

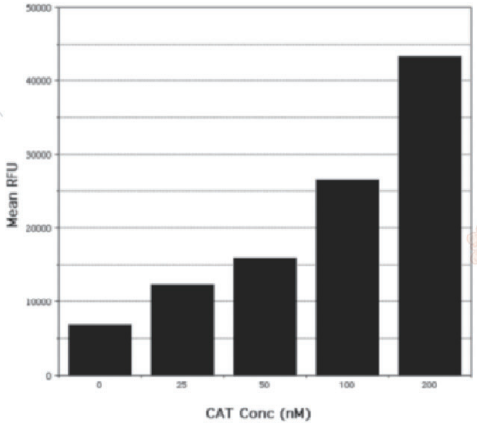

Figure 1: Typical endpoint assay with Chloramphenicol acetyltransferase (CAT) titrated against 100 uM of chloramphenicol (substrate). Serial dilutions of the enzyme were prepared in Transferase Assay buffer. Based on this titration data, the acetyltransferase concentration of 100nm produces a maximum signal within the detection range of the plate reader, with a signal to noise ratio sufficient for easy detection of altered enzyme activity.

TYPICAL KINETIC ASSAY RESULTS

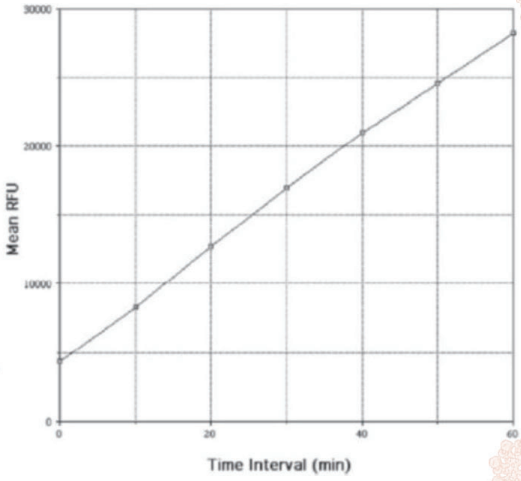

Figure 2: Typical kinetic assay using 100 nM chloramphenicol acetyltransferase (CAT) and 100 µM chloramphenicol substrate.

TYPICAL INHIBITION ASSAY RESULTS

The results shown below are for illustration only and **should not** be used to calculate results from another assay.

Dilution	Inhib conc (mM)	Mean RFU	Mean Net RFU	% Inhibition
1	100	8707	2161	93.6
2	50	15575	9029	73.1
3	25	24951	18405	45.2
4	12.5	30110	23564	29.8
0	0	40127	33581	0
Blank	---	6889	0	---

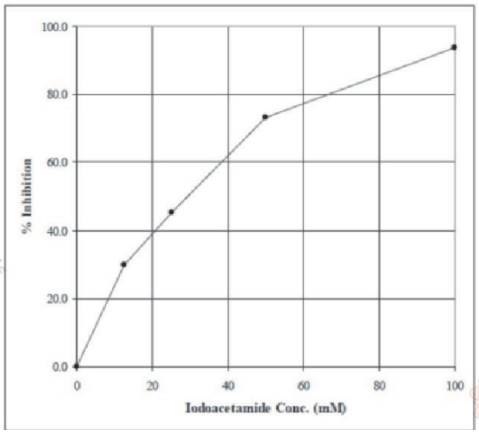

Figure 3: Typical inhibition curve using chloramphenicol acetyltransferase and chloramphenicol as an enzyme substrate system**.** Percent inhibition for dilutions of iodoacetamide were tested.

17. 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 1X Transfer Assay Buffer, 1 X Transfer Detection Solution Acetyltransferase positive control and 1X Reaction Mix; get equipment ready.
- Prepare samples in duplicate (make serial dilution of test samples in the assay buffer).

ENDPOINT ASSAY

- Set up the plate as follows:

Component	Test sample well (µL)	Blank wells (µL)	Positive control wells (µL)	Blank for positive control (µL)
1X Transferase Assay Buffer		25	0	50
Acetyltransferase dilutions	25		0	0
1X Reaction Mix	25	25	0	0
1X Positive Control	0	0	50	0

Cover plate with plate sealer. Incubate for 30 minutes shaking

Component	Test sample well (µL)	Blank wells (µL)	Positive control wells (µL)	Blank for positive control (µL)
Isopropyl alcohol	50	50	50	50
1X Detection Solution	100	100	100	100

Incubate for 10 min, RT, sealed

- Read fluorescence at Ex/Em= 380/520nm.

KINETIC ASSAY

- Set up the plate as follows:

Component	Enzyme sample well (µL)	Blank wells (µL)
1X Transferase Assay Buffer	0	25
Acetyltransferase dilutions	25	
1X Reaction Mix	25	25

- Cover plate with plate sealer. Incubate for 30 minutes shaking
- Stop replicate dilutions at predetermined intervals with 50 µL ice cold isopropyl alcohol.

Component	Enzyme sample well (µL)	Blank wells (µL)
1X Detection Solution	100	100

- Incubate for 10 min, RT, sealed
- Read fluorescence at Ex/Em= 380/520 nm.

INHIBITION ASSAY

- Prepare 1X Transfer Assay Buffer, 1 X Transfer Detection Solution (Section 9.3), and 1X Reaction Mix; get equipment ready.

Component	Blank well (µL)	Inhibition samples (µL)	Zero well (µL)
1X Transferase Assay Buffer	25		10
Inhibitor sample dilution		10	
Acetyltransferase		15	15

- Incubate for 10 min, RT, sealed
- Add 25 µL 1X Reaction Mix to blank, Inhibition Sample and zero wells.
- Cover plate with plate sealer. Incubate for 30 min, RT, shaking

Component	Blank well (µL)	Inhibition samples (µL)	Zero well (µL)
Isopropyl alcohol	50	50	50
1X Detection Solution	100	100	100

- Incubate for 10 min, RT, sealed.
- Read fluorescence at Ex/Em=380/520 nm

18.TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19.INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure.

• Be sure no sodium azide is present in this assay, as this inhibits HRP enzyme activity.

INTERFERENCE –

The following solvents were tested for interference with the fluorescent signal generated in the assay. The table lists the percentage of signal in the presence of interferant relative to the zero for each solvent.

Interfering Substances

% Interferant	DMSO	DMF	Acetonitrile
12.5	108	68	49
6.25	107	82	71
3.12	107	88	86
1.56	112	98	96
0.78	114	104	95
0.39	115	109	100
0	100	100	100

* Percent interferant is relative to a 50 µL total reaction volume.

Diluents containing bovine serum albumin (BSA), or other thiol containing reagents, should be treated with N-Ethylmaleimide (NEM) prior to use in the assay. We recommend reacting 10% BSA with 1 mM NEM for 1 hour at room temperature. However this procedure should be optimized for each reagent.

For research use only! Not to be used in humans.

For more details, please visit http://www.apexbt.com/or contact our technical team.

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