

# PicoProbe™ Acetyl CoA Assay Kit

(Catalog #GWB-AXR197; Store Kit at -20°C)

## I. Introduction:

Acetyl CoA is a central molecule of metabolism. It carries acetate, used in the build-up and breakdown of larger molecules. Acetyl CoA is key in synthetic pathways leading to sesquiterpenes, precursors to cholesterol and other sterols, flavonoids and other polyketides, polyenes and long-chain fatty acids. It is the source of the acetyl group used in histone acetylation. The acetyl group is also incorporated into a variety of other molecules such as acetylcholine, melatonin, heme and TCA cycle intermediates. GenWay Biotech has developed a highly sensitive assay for determining Acetyl CoA level in a variety of biological samples. In

Components	GWB-AXR197	Cap Code	Part Number
Acetyl CoA Assay Buffer	25 ml	WM	GWB-AXR197-1
PicoProbe	0.2 ml	Blue	GWB-AXR197-2
Conversion Enzyme	0.1 ml	Green	GWB-AXR197-3
Acetyl CoA Enzyme Mix	0.5 ml	Purple	GWB-AXR197-4
Acetyl CoA Substrate Mix	lyophilized	Red	GWB-AXR197-5
CoA Quencher	1.0 ml	Orange	GWB-AXR197-6
Quench Remover	lyophilized	Clear	GWB-AXR197-7
Acetyl CoA Standard (1 μmol)	lyophilized	Yellow	GWB-AXR197-8

the assay, free CoA is quenched then Acetyl CoA is converted to CoA. The CoA is reacted to form NADH which interacts with PicoProbe to generate fluorescence (Ex/Em =535/587 nm). The assay can detect 10 to 1000 pmol of Acetyl CoA (with detection limit ~ 0.4 μM) in a variety of samples.

## II. Kit Contents:

### III. Storage and Handling:

Store kit at -20°C, protect from light. Warm Acetyl CoA Assay Buffer to room temperature prior to using it. Briefly centrifuge all small vials prior to opening.

### IV. Reagent Preparation and Storage Conditions:

**PicoProbe:** in DMSO, ready to use as supplied. Thaw by warming to room temperature. Mix well, store at -20°C.

**Substrate Mix:** Dissolve with 220 μl Assay Buffer. Pipette up and down to completely dissolve. Store at -20°C. Use within two months.

**Quench Remover:** Dissolve in 220 μl dH<sub>2</sub>O. Keep on ice while in use, store at -20°C.

**Acetyl CoA Standard:** Dissolve in 100 μl dH<sub>2</sub>O to generate 10 mM (10 nmol/μl) Acetyl CoA Standard solution. Keep cold while in use. Store at -20°C.

## V. Acetyl CoA Assay Protocol:

### 1. Acetyl CoA Standard Curve Preparations:

**0 - 1 nmol Range:** Dilute the Acetyl CoA Standard 100X to 0.1 mM (100 pmol/μl) by taking 10 μl into 990 μl dH<sub>2</sub>O. Dilute a further 5X to 0.02 mM by adding 100 μl to 400 μl dH<sub>2</sub>O. Add 0, 10, 20, 30, 40, 50 μl into a series of wells in a 96-well plate. Adjust volume to 50 μl/well with dH<sub>2</sub>O to generate 0, 200, 400, 600, 800, 1000 pmol/well Acetyl CoA standard.

**0 - 100pmol Range:** Dilute the Acetyl CoA Standard 100X to 0.1 mM (100 pmol/μl) by taking 10 μl into 990 μl dH<sub>2</sub>O. Dilute an additional 50X to 2 μM (2 pmol/μl) by taking 10 μl into 490 μl of dH<sub>2</sub>O. Mix well. Add 0, 10, 20, 30, 40, 50 μl into a series of standards wells on a 96 well plate. Adjust volume to 50 μl/well with dH<sub>2</sub>O to generate 0, 20, 40, 60, 80, 100 pmol/well Acetyl CoA standard.

**Sample Preparation:** Enzymes in samples interfere with the assay. You should deproteinize your sample using a perchloric acid/KOH protocol (GenWay Cat. #GWB-AXR197). Tissue

samples (20 - 1000 mg) should be frozen rapidly (liquid N<sub>2</sub> or methanol/dry ice), weighed and pulverized.

Add 2 μl 1N perchloric acid/mg sample. KEEP COLD! Homogenize or sonicate thoroughly.

Spin homogenate at 10,000 x g. Neutralize supernatant with 3 M KHCO<sub>3</sub>, adding repeated 1 μl aliquots/10 μl supernatant while vortexing until bubble evolution ceases (2 - 5 aliquots). Put on ice for 5 min. Check pH (using 1 μl) should be ~ pH 6 - 8. Spin 2 min to pellet KClO<sub>4</sub>. Add 10 μl of sample into duplicate wells (Sample and Background) of a 96-well plate; bring volume to 50 μl with Assay Buffer.

2. Free CoASH and succ-CoA in samples generate background. In order to correct for this background, add 10 μl of CoASH Quencher to each Standard, Sample and background sample to quench free CoA. Incubate for 5 min at room temp. Then add 2 μl of Quench Remover, mix and incubate 5 min. In addition, run background control for each sample to correct for succ-CoA for some other forms by omitting the Conversion Enzyme.

3. **CoA Conversion:** Make up 50 μl of reaction mix for each well to be tested (Standard, Sample and Background):

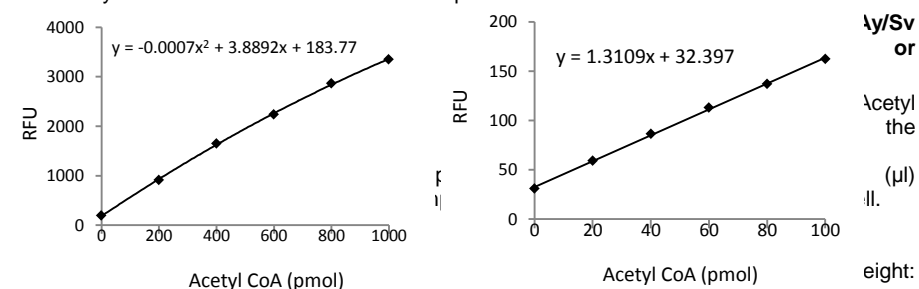
	0 - 1 nmol	Bkgd	0-100 pmol	Bkgd
Buffer:	40 μl	41 μl	41.8 μl	42.8 μl
Substrate Mix:	2 μl	2 μl	2 μl	2 μl
Conversion Enzyme:	1 μl	----	1 μl	----
Enzyme Mix:	5 μl	5 μl	5 μl	5 μl
PicoProbe:	2 μl	2 μl	0.2 μl	0.2 μl

4. **Incubate** for 10 min at 37°C.

5. Measure fluorescence using Ex/Em = 535/589 nm with a plate reader.

6. **Calculation:** Correct background by subtracting the value of the 0 Acetyl CoA Standard from all readings (Note: The background reading can be significant and must be subtracted from sample readings). Determine Background values for each sample tested and correct Acetyl CoA values for this background. Plot the Standard Curve. Apply the sample readings to the Standard Curve to get the Acetyl CoA amount in the sample wells.

The Acetyl CoA concentrations in the test samples:



Standard curves were generated following this kit protocol.

**Note:** White plates enhance the sensitivity of fluorescent assays and are highly recommended

**GENERAL TROUBLESHOOTING GUIDE:**

<b>Problems</b>	<b>Cause</b>	<b>Solution</b>
Assay not working	<ul style="list-style-type: none"> <li>• Use of ice-cold assay buffer</li> <li>• Omission of a step in the protocol</li> <li>• Plate read at incorrect wavelength</li> <li>• Use of a different 96-well plate</li> </ul>	<ul style="list-style-type: none"> <li>• Assay buffer must be at room temperature</li> <li>• Refer and follow the data sheet precisely</li> <li>• Check the wavelength in the data sheet and the filter settings of the instrument</li> <li>• Fluorescence: Black plates or white plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates</li> </ul>
Samples with erratic readings	<ul style="list-style-type: none"> <li>• Use of an incompatible sample type</li> <li>• Samples prepared in a different buffer</li> <li>• Samples were not deproteinized (if indicated in datasheet)</li> <li>• Cell/ tissue samples were not completely homogenized</li> <li>• Samples used after multiple free-thaw cycles</li> <li>• Presence of interfering substance in the sample</li> <li>• Use of old or inappropriately stored samples</li> </ul>	<ul style="list-style-type: none"> <li>• Refer data sheet for details about incompatible samples</li> <li>• Use the assay buffer provided in the kit or refer data sheet for instructions</li> <li>• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated</li> <li>• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope</li> <li>• Aliquot and freeze samples if needed to use multiple times</li> <li>• Troubleshoot if needed, deproteinize samples</li> <li>• Use fresh samples or store at correct temperatures till use</li> </ul>
Lower/ Higher readings in Samples and Standards	<ul style="list-style-type: none"> <li>• Improperly thawed components</li> <li>• Use of expired kit or improperly stored reagents</li> <li>• Allowing the reagents to sit for extended times on ice</li> <li>• Incorrect incubation times or temperatures</li> <li>• Incorrect volumes used</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw all components completely and mix gently before use</li> <li>• Always check the expiry date and store the components appropriately</li> <li>• Always thaw and prepare fresh reaction mix before use</li> <li>• Refer datasheet &amp; verify correct incubation times and temperatures</li> <li>• Use calibrated pipettes and aliquot correctly</li> </ul>
Readings do not follow a linear pattern for Standard curve	<ul style="list-style-type: none"> <li>• Use of partially thawed components</li> <li>• Pipetting errors in the standard</li> <li>• Pipetting errors in the reaction mix</li> <li>• Air bubbles formed in well</li> <li>• Standard stock is at an incorrect concentration</li> <li>• Calculation errors</li> <li>• Substituting reagents from older kits/ lots</li> </ul>	<ul style="list-style-type: none"> <li>• Thaw and resuspend all components before preparing the reaction mix</li> <li>• Avoid pipetting small volumes</li> <li>• Prepare a master reaction mix whenever possible</li> <li>• Pipette gently against the wall of the tubes</li> <li>• Always refer the dilutions in the data sheet</li> <li>• Recheck calculations after referring the data sheet</li> <li>• Use fresh components from the same kit</li> </ul>
Unanticipated results	<ul style="list-style-type: none"> <li>• Measured at incorrect wavelength</li> <li>• Samples contain interfering substances</li> <li>• Use of incompatible sample type</li> <li>• Sample readings above/below the linear range</li> </ul>	<ul style="list-style-type: none"> <li>• Check the equipment and the filter setting</li> <li>• Troubleshoot if it interferes with the kit</li> <li>• Refer data sheet to check if sample is compatible with the kit or optimization is needed</li> <li>• Concentrate/ Dilute sample so as to be in the linear range</li> </ul>

**Note:** The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.