DATA SHEET

For research use only

Acetyl-CoA Assay Kit

(Catalog #K377-100; 100 assays; Store Kit at -20°C)

I. Introduction:

Acetyl-CoA(AcCoA) is the metabolic intermediate which transfers carbon atoms to the TCA cycle for energy production and is the link between fat and carbohydrate metabolism. When fatty acid levels are high, AcCoA levels rise beyond the energy requirements of the organism and the excess is converted to the ketone bodies acetone and β-hydroxybutyrate. AcCoA also contributes carbon such as histone acetylation and isoprenoid synthesis. BioVision has developed an easy, convenient assay to measure the AcCoA level in biological samples. In the assay, AcCoA is specifically utilized to generate products which react with OxiRed Probe to generate color (λ=570 nm) and fluorescence (Ex/Em=535/587 nm). The assay can detect 0.1 to 10 nmol of AcCoA in a variety of samples.

II. Kit Contents:

Components	K367-100	Cap Code	Part Number
AcCoA Assay Buffer	25 ml	WM	K377-100-1
OxiRed Probe	lyophilized	Red	K377-100-2
DMSO (anhydrous)	0.5 ml	Brown	K377-100-3
Conversion Enzyme Mix	lyophilized	Blue	K377-100-4
AcCoA Substrate	1 ml	Purple	K377-100-5
AcCoA Developer	lyophilized	Green	K377-100-6
AcCoA Standard (10 μmol)	lyophilized	Yellow	K377-100-7

III. Storage and Handling:

Store kit at –20°C, protect from light. Warm AcCoA Assay Buffer to room temperature before use. Briefly centrifuge all small vials prior to opening.

IV. Reagent Preparation and Storage Conditions:

OxiRed Probe: Dissolve with 220 μl of DMSO (provided, need to warm up >18°C to become liquid). Mix well, store at –20°C, protect from light and moisture.

Conversion Enzyme Mix, AcCoA Developer: Dissolve with 220 μl AcCoA Assay Buffer. Pipette up and down to completely dissolve. Store at –20°C. Use within two months.

Substrate: Ready to use as supplied.

AcCoA Standard: Dissolve in 100 μl dH2O to generate 100 mM (100 nmol/μl) AcCoA Standard solution. Keep cold while in use. Store at -20°C.

V. AcCoA Assay Protocol:

1. AcCoA Standard Curve Preparations:

Colorimetric assay: Dilute the AcCoA Standard to 1 nmol/μl by adding 10 μl of the Standard to 990 μl of dH2O, mix well. Add 0, 2, 4, 6, 8, 10 μl into a series of standards wells on a 96 well plate. Adjust volume to 40 μl/well with Assay Buffer to generate 0, 2, 4, 6, 8, 10 nmol/well of the Standard.

Fluorometric assay: Dilute the AcCoA Standard to 1 nmol/μl as for the colorimetric assay. Then dilute another 10-fold to 0.1 nmol/μl by taking 10 μl into 90 μl of dH2O. Mix well. Add 0, 4, 8, 12, 16, 20 μl into a series of standards wells on a 96 well plate. Adjust volume to 40 μl/well with Assay Buffer to generate 0, 0.4, 0.8, 1.2, 1.6, 2.0 nmol/well of the standard.

2. Sample Preparation:

Tissue samples (100 mg) should be rapidly homogenized with 100 μl ice cold PBS or other buffer (pH 6.5-8). Enzymes in samples may interfere with the assay. We suggest deproteinizing your sample using a perchloric acid/KOH protocol (BioVision, Cat. #K808-200) or 10 kd molecular weight cut off spin columns (BioVision, Cat # 1997-25). Add 1-40 μl sample into 96-well plate, bring volume to 40 μl with Assay Buffer. We suggest testing several doses of your samples to ensure the readings are within the standard curve range.

3. AcCoA Conversion:

Add 8 μl of Substrate, 2 μl of Conversion Enzyme Mix* to each standard and sample. Mix well.

*Long chain acyl-CoA's in the sample can generate background in the assay. If significant amount of acyl-CoA is in your sample, do a background control; omit Conversion Enzyme from the reaction. The acyl-CoA background should be subtracted from CoA readings.

4. Incubate for 30 minutes at 37°C.

5. Develop:

Mix enough reagent for the number of samples and standards to be performed: For each well, prepare a total 50 μl Reaction Mix containing:

	Colorimetric	Fluorometric
AcCoA Assay Buffer	46 μl	46 μl
AcCoA Developer	2 μl	1 μl
OxiRed Probe	2 μl	3 μl**

Add 50 μl of the Reaction Mix to each well containing the AcCoA Standard and test samples.

** Dilute OxiRed Probe 1:10 in the fluorometric assay to decrease fluorescence background and thus increase detection sensitivity, significantly.

6. Incubate for 30 minutes at 37°C, protect from light.

7. Measure OD at 570 nm for the colorimetric assay, or Ex/Em=535/589 for the fluorometric assay.

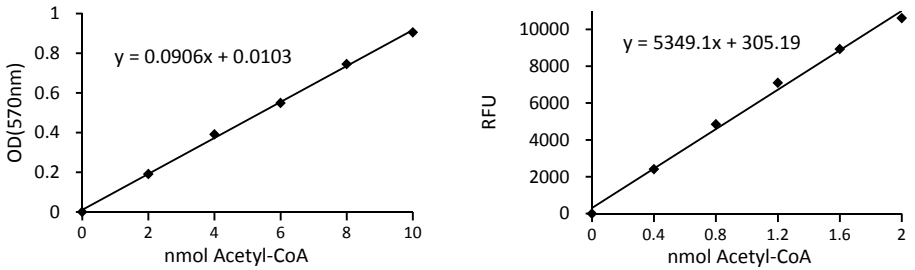
8. Calculation:

Correct background by subtracting the value of the 0 AcCoA control from all sample readings (Note: The background reading can be significant and must be subtracted from sample readings). Plot the standard curve. Then apply the sample readings to the standard curve to get AcCoA amount in the sample wells.

The AcCoA concentrations in the test samples:

C = Ay/Sv (nmol/μl; or μmol/ml; or mM)

Where: Ay is the amount of Acetyl-CoA (nmol) in your sample from the standard curve.
Sv is the sample volume (μl) added to the sample well.
Acetyl-CoA molecular weight: 809.57.



Acetyl-CoA standard curves generated following this kit instructions

VI. RELATED PRODUCTS:

Apoptosis Detection Kits & Reagents
Glucose and Sucrose Assay Kit
Glutathione Assay Kit
NAD/NADH and NADP/NADPH Assay Kit
TAC Total Antioxidant Capacity
Cell Proliferation & Senescence Kits
Cholesterol, LDL/HDL Assay Kits
Ethanol and Uric Acid Assay Kit
Lactate Assay Kits
Mono or Polysaccharide Assay Kits

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium