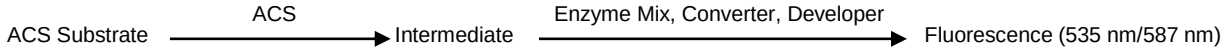
Acyl-CoA Synthetase Fluorometric Assay Kit

05/19

(Catalog # K184-100; 100 assays; Store at -20°C)

I. Introduction:

Acyl-CoA Synthetase (ACS) also known as Acyl-CoA ligase, is a ubiquitous enzyme that catalyzes the reaction of free fatty acids and Coenzyme A (CoA) to generate Fatty Acyl-CoAs through an ATP-dependent mechanism. The incorporation of this CoA moiety generates a thio-ester bond that effectively activates the fatty acid for downstream biological processes. Unlike their fatty acid counterparts, Fatty acyl-CoAs also serve as precursors of cellular lipids. Dysregulation of the various isoforms of ACS have been implicated in a number of metabolic diseases, including Non-Alcoholic Fatty Liver Disease and Hepatic Fibrosis. ACS also plays a role in regulating apoptosis. BioVision's Acyl-CoA Synthetase Fluorometric assay kit provides a rapid, sensitive and straightforward way to measure ACS activity in various samples. In the assay, acyl-CoA produced by ACS activity is metabolized by the Enzyme Mix, Developer Mix and Converter Mix to generate an intermediate compound, which reacts with a probe, yielding a fluorescent signal that can be measured with excitation at 535 nm and emission at 587 nm. This assay can detect ACS activity as low as 5 mU/µl sample.



II. Applications:

- Measurement of Acyl-CoA Synthetase activity in various tissues/cells

III. Sample Type:

- Animal tissues: Liver, intestine etc.
- Purified enzyme preparations
- Cell culture: Adherent or suspension cells

IV. Kit Contents:

Components	K184-100	Cap Code	Part Number
ACS Assay Buffer	25 ml	WM	K184-100-1
ACS Substrate	1.1 ml	Purple	K184-100-2
ACS Enzyme Mix	1 vial	Green	K184-100-3
ACS Converter	1 vial	White	K184-100-4
ACS Developer	1 vial	Blue	K184-100-5
ACS Positive Control	1 vial	Orange	K184-100-6
ACS Probe	0.2 ml	Red	K184-100-7
H_2O_2 Standard	100 µl	Yellow	K184-100-8

V. User Supplied Reagents and Equipment:

- 96-well black plate with flat bottom
- Multiwell fluorescence microplate reader

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **ACS Assay Buffer:** Warm to room temperature (RT) before use. Store at -20ºC. Use within two months.
- **ACS Substrate, H_2O_2 Standard, and ACS Probe:** Ready-to-use as supplied. Thaw and warm to RT.
- **ACS Enzyme Mix:** Reconstitute with 220 µl ACS Assay Buffer, pipet up and down to mix. Store at –20°C, protected from light. Use within two months.
- **ACS Positive Control:** Reconstitute with 100 µl ACS Assay Buffer, pipet up and down to mix. Aliquot and store at –20°C, protected from light. Use within two months.
- **ACS Converter and ACS Developer:** Reconstitute with 220 µl ddH_2O. Aliquot and store at -20°C. Use within two months.

VII. Acyl-CoA Synthetase Assay Protocol:

1. Sample Preparation: Rapidly homogenize tissue (10 mg) or cells (1 x 10^6) with 100 µl ice cold ACS Assay Buffer. Centrifuge at 10,000 x *g* and 4ºC for 15 min and transfer the supernatant to a fresh tube. Since any given Sample may produce Background signal, you will need to designate two wells of a 96-well black plate (Sample & Sample Background) for each concentration of the Sample. Add the same desired volume (2-20 µl) of the Sample supernatant to both the wells. Adjust the volume to 50 µl/well with ACS Assay Buffer.

Positive Control: Add 2-4 µl Positive Control to Positive Control well(s). Adjust the volume to 50 µl/well with ACS Assay Buffer.

Notes:

a. For Unknown Samples, we suggest testing several volumes to ensure the readings are within the Standard Curve range.

b. If too much ACS activity is present in the well, the assay kinetics will not be linear for more than a minute or two. In this case, additional dilution of the Sample is recommended.

2. H_2O_2 Standard Curve: Dilute 10 µl H_2O_2 Standard with 870 µl dH_2O to generate 10 mM H_2O_2 Standard. Dilute 10 µl of the 10 mM H_2O_2 Standard into 990 µl dH_2O to generate a 0.1 mM H_2O_2 Standard. Add 0, 2, 4, 6, 8, and 10 µl of the 0.1 mM H_2O_2 Standard into a 96-well plate in duplicates to generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well H_2O_2 Standard. Bring the volume in each well to 50 µl with ACS Assay Buffer.

3. Reaction Mix:

	Reaction Mix	Background Control Mix
ACS Assay Buffer	41.6 µl	43.6µl
ACS Substrate	2 µl	----
ACS Enzyme Mix	2 µl	2 µl
ACS Converter	2 µl	2 µl
ACS Developer	2 µl	2 µl
ACS Probe	0.4 µl	0.4 µl

Mix well. Add 50 µl of the Reaction Mix into Standard and Sample wells. Add 50 µl of the Background Control Mix into Sample Background well(s). The Sample Background reading should be subtracted from all Sample readings.

4. Measurement: Measure fluorescence (Ex/Em= 535/587 nm) in a kinetic mode for 30 min at 37°C. The Standard Curve should reach the endpoint (maximal signal) within 10 min.

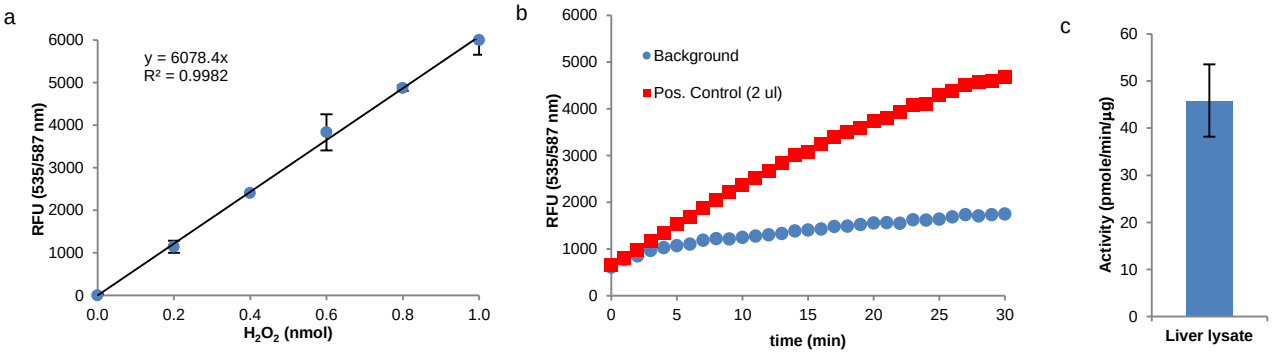
Note: Measurement time for the linear phase of the reaction depends on the ACS activity in Samples. Measure the fluorescence in kinetic mode and choose any two time points (t_1 and t_2) in the linear range to calculate the ACS activity of the Sample(s). Do NOT use the first 5 min of the reaction for activity calculations.

5. Calculation: Subtract the 0 nmol Standard reading from all Standard readings. Plot the H_2O_2 Standard Curve. Subtract the Sample Background reading from all Sample readings within t_1 and t_2. Apply the $\Delta RFU = RFU_1 - RFU_2$ to the Standard Curve to get B nmol of H_2O_2 generated during the reaction time ($\Delta t = t_2 - t_1$). Sample Acyl-CoA Synthetase Activity is calculated as:

Sample Acyl-CoA Synthetase Activity = B/(Δt X V) x D = nmol/min/ml = mU/ml

Where: **B** = H_2O_2 amount from the Standard Curve (nmol)
Δt = reaction time (min)
V = Sample volume added into the reaction well (ml)
D = Dilution Factor (For Undiluted Samples, D=1)

Unit Definition: One unit of **Acyl-CoA Synthetase** is the amount of enzyme that generates 1.0 µmole of Umbelliferone per min at pH 7.5 at 37°C.



Figures: (a) H_2O_2 Standard Curve. (b) Reaction kinetics of ACS Positive Control. (c) ACS activity in Rat liver lysate. Rat liver was prepared according to the described protocol. For activity determination, experiments were run in duplicates. 3.3-13.3 µg protein was loaded per well.

VIII. RELATED PRODUCTS:

Arachidonic Acid (1505)
Triglyceride Quantification Assay Kit (K622)
Cholesterol/Cholesteryl Ester Quantitation Kit (K603)
Free Fatty Acid Quantification Colorimetric/Fluorometric Kit (K612)
HDL and LDL/VLDL Quantitation Kit (K613)
JZL195 (B1064)
PicoProbe™ Triglyceride Fluorometric Assay Kit (K614)
Cholesterol/Cholesteryl Ester Quantitation Assay Kit II (K623)
EZScreen™ Triglyceride Assay Kit-384 Well Format (K952)
Cyclooxygenase (COX) Activity Assay Kit (Fluorometric) (K549)

FOR RESEARCH USE ONLY! Not to be used on humans.