TECHNICAL MANUAL

Acyl-CoA Synthetase (ACS) Activity Colorimetric Assay Kit

- **SKU CODE:** MAES0519
- **SIZE:** 96 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Incubate and measure absorbance at 555 nm

2. Intended use

This kit can be used to measure acyl-CoA synthetase (ACS) activity in serum, plasma, animal tissue, plant tissue, and cell samples.

3. Detection principle

Acyl-CoA synthase (ACS) is an important enzyme that is widely present in living organisms and participates in the metabolic process of fatty acids. It is an enzyme necessary for lipid synthesis, fatty acid catabolism, and membrane remodeling, and can also function as a transcriptional activator, allosteric inhibitor, or precursor of inflammatory mediators. In the medical field, mutations or abnormal expression of ACS may be associated with various diseases, such as diabetes and obesity.

The detection principle of this kit: ACS catalyzes the substrate, and the generated product reacts with the chromogenic agent to produce a purple substance, which has the largest absorption peak at 555 nm. Therefore, the absorbance at 555 nm can be measured to calculate the amount of product generated, thereby calculating the activity of ACS.

4. Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	50 mL x 1 vial	-20°C, 12 months, protect from light
Reagent 2	Co-factor	Powder x 2 vials	-20°C, 12 months, protect from light
Reagent 3	Accelerant	Powder x 2 vials	-20°C, 12 months, protect from light

Item	Component	Size (96 T)	Storage
Reagent 4	Substrate	0.3 mL x 1 vial	-20°C, 12 months, protect from light
Reagent 5	Enzyme Reagent	3.6 mL x 1 vial	-20°C, 12 months, protect from light
Reagent 6	Chromogenic Agent	3.6 mL x 1 vial	-20°C, 12 months, protect from light
Reagent 7	2 mmol/L Standard Solution	1.6 mL x 1 vial	-20°C, 12 months, protect from light
	Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (550-560 nm, optimum wavelength: 555 nm), Incubator

6. Reagent preparation

1. Equilibrate all reagents to 25°C before use.
2. **Preparation of co-factor working solution::** Dissolve one vial of co-factor with 0.8 mL of buffer solution, mix well to dissolve. Store aliquoted at -20°C for 7 days protected from light.
3. **Preparation of accelerant working solution::** Dissolve one vial of accelerant with 2.7 mL of buffer solution, mix well to dissolve. Store aliquoted at -20°C for 7 days protected from light.
4. **Preparation of reaction working solution::** Before testing, prepare sufficient reaction working solution according to the test wells. For example, prepare 60 µL of reaction working solution (mix well 10 µL of co-factor working solution and 50 µL of accelerant working solution). Keep on ice during use protected from light. Store at -20°C for 2 days protected from light.
5. **Preparation of substrate working solution::** Incubate the substrate at 37°C for 10 min. Before testing, prepare sufficient substrate working solution according to the test wells. For example, prepare 100 µL of substrate working solution (mix well 5

μL of substrate and 95 μL of double distilled water). Keep on ice during use protected from light. Store at -20°C for 2 days protected from light.

6. **Preparation of chromogenic working solution::** For each well, prepare 60 μL of chromogenic working solution (mix well 30 μL of enzyme reagent and 30 μL of chromogenic agent). Keep on ice during use protected from light. Store at -20°C for 2 days protected from light.
7. **Preparation of standard curve::** Always prepare a fresh set of standards. Discard working standard dilutions after use. Dilute 2 mmol/L standard with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 0.2, 0.4, 0.8, 1, 1.2, 1.6, 2 mmol/L.

7. Standard curve preparation

Item	1	2	3	4	5	6	7	8
Concentration (mmol/L)	0	0.2	0.4	0.8	1	1.2	1.6	2
2 mmol/L standard (μL)	0	20	40	80	100	120	160	200
Buffer solution (μL)	200	180	160	120	100	80	40	0

8. Sample preparation

Sample preparation

Serum or plasma samples: Detect directly.

Tissue sample:

1. Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

2. Homogenize 20 mg tissue in 180 μL buffer solution with a dounce homogenizer at 4°C.

3. Centrifuge at 10,000 x g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection. The supernatant should be used within 4 hours.

Cell (adherent or suspension) samples:

1. Harvest the number of cells needed for each assay (initial recommendation 1 x 10^6 cells).

2. Homogenize 1 x 10^6 cells in 200 µL buffer solution with an ultrasonic cell disruptor at 4°C.

3. Centrifuge at 10,000 x g for 10 min at 4°C to remove insoluble material. Collect supernatant and keep on ice for detection. The supernatant should be used within 4 hours.

Dilution of sample

The recommended dilution factor for different samples is as follows (for reference only):

Human plasma: 1

Mouse serum: 1

10% Mouse liver tissue homogenate: 1-4

10% Mouse kidney tissue homogenate: 1

10% Mouse brain tissue homogenate: 1

10% Mouse heart tissue homogenate: 1

10% Mouse lung tissue homogenate: 1

10% Mouse leg muscle tissue homogenate: 1

10% Mouse spleen tissue homogenate: 1

10% Corn seed tissue homogenate: 1

1 x 10^6 Molt-4 cells: 1

Note: The diluent is buffer solution. For the dilution of other sample types, please perform a pretest to confirm the dilution factor.

9. The key points of the assay

It is necessary to observe whether the substrate is clarified before use. If it is not completely clarified after 10 min at 37°C, the incubation time can be extended appropriately.

10. Operating steps

1. Standard well: Add 10 µL of standard with different concentrations into wells. Sample well: Add 10 µL of sample into wells. Control well: Add 10 µL of sample into wells.

2. Add 100 µL of buffer solution into standard wells. Add 50 µL of reaction working solution into sample and control wells.
3. Add 50 µL of double distilled water into control wells. Add 50 µL of substrate working solution into sample wells.
4. Add 60 µL of chromogenic working solution into each well.
5. Mix fully with microplate reader for 5 s and incubate at 37°C for 10 min protected from light. Measure the OD value of each well at 555 nm.

11. Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absolute OD value.

3. Plot the standard curve by using absolute OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (y = ax + b) with graph software (or Excel).

The sample:

1. Serum and plasma samples:

Definition: The amount of enzyme in 1 L serum or plasma per 1 min that produces 1 mmol of H_{2O}_2 at 37°C is defined as 1 unit.

ACS activity (U/L) = (ΔA_555 - b) ÷ a ÷ T × f

2. Tissue samples:

Definition: The amount of enzyme in 1 kg tissue per 1 min that produces 1 mmol of H_{2O}_2 at 37°C is defined as 1 unit.

ACS activity (U/kg wet weight) = (ΔA_555 - b) ÷ a ÷ T ÷ m × v × f

3. Cell samples:

Definition: The amount of enzyme in 1 × 10^6 cells per 1 min that produces 1 µmol of H_{2O}_2 at 37°C is defined as 1 unit.

ACS activity (U/10^6) = (ΔA_555 - b) ÷ a ÷ T ÷ n × v × f

[Note]

ΔA_555: OD_{sample} – $OD_{control}$.

T: Reaction time, 10 min.

m: The wet weight of sample, kg.

n: The number of cell sample/10^6.

V: The volume of buffer solution in the preparation step of sample, L.

f: Dilution factor of sample before test.

12. Appendix

Performance Characteristics

Intra-assay Precision

Three human plasma samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Intra-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.02	0.04	0.08
%CV	3.6	2.7	2.0

Inter-assay Precision

Three human plasma samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Inter-assay Precision Data

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.02	0.04	0.08
%CV	8.9	6.8	3.8

Recovery

Three samples of high concentration, middle concentration, and low concentration were tested with each concentration run 6 times in parallel to obtain the average recovery rate of 97.7%.

Recovery Data

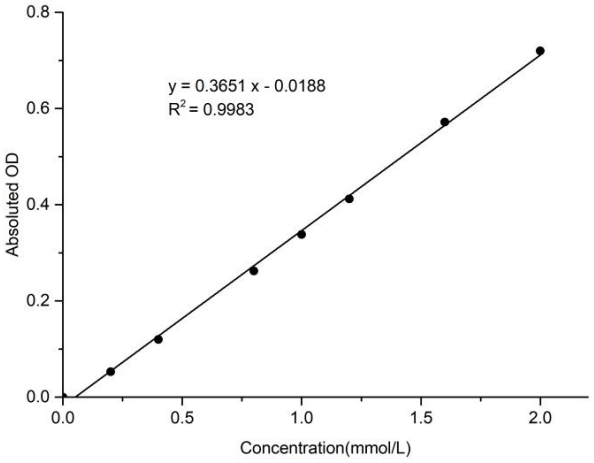
	Sample 1	Sample 2	Sample 3
Expected Conc. (U/L)	0.02	0.04	0.08
Observed Conc. (U/L)	0.020	0.040	0.076
Recovery rate (%)	98.0	100.0	95.0

Sensitivity

The analytical sensitivity of the assay is 0.004 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

Standard curve

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g., operator, pipetting technique, or temperature effects), the standard curve and data are provided below for reference only:



Standard curve data

Concentration (mmol/L)	OD value 1	OD value 2	Average OD value	Absolute OD value
0	0.058	0.058	0.058	0
0.2	0.108	0.114	0.111	0.053
0.4	0.176	0.180	0.178	0.120

Concentration (mmol/L)	OD value 1	OD value 2	Average OD value	Absolute OD value
0.8	0.318	0.322	0.320	0.262
1	0.391	0.401	0.396	0.338
1.2	0.470	0.472	0.471	0.412
1.6	0.627	0.635	0.631	0.572
2	0.778	0.778	0.778	0.720

Example Analysis

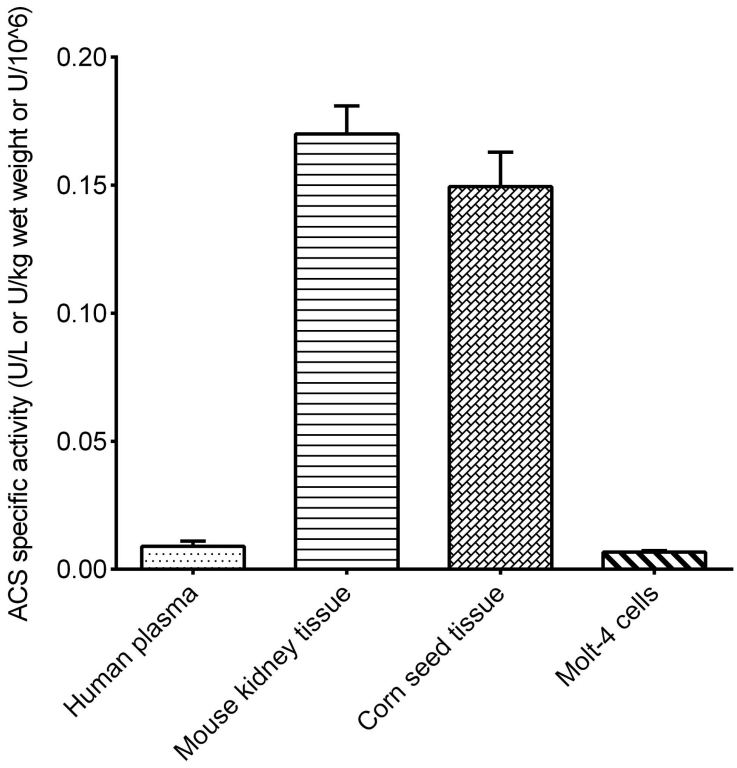
Example analysis:

Take 10 µL of 10% mouse kidney tissue homogenate and carry out the assay according to the operation steps. The results are as follows:

Standard curve: y = 0.3651x - 0.0188, the average OD value of the sample well is 0.136, the average OD value of the control well is 0.086, $\Delta A_555 = OD_{sample} – OD_{control} = 0.136 – 0.086 = 0.050$, and the calculation result is:

ACS activity (U/kg wet weight) = (0.050 + 0.0188) ÷ 0.3651 ÷ 10 ÷ 0.0001 × 0.0009 = 0.170 U/kg wet weight

Detect human plasma, 10% mouse kidney tissue homogenate, 10% corn seed tissue homogenate and 1×10^6 Molt-4 cells, according to the protocol, the result is as follows:



13. Statement

1. This assay kit is for Research Use Only. Assay Genie assumes no responsibility for any problems or legal liabilities arising from the use of this kit for clinical diagnosis or any other purpose.

2. Please read the instructions carefully and calibrate the instruments before performing the experiments. Follow the instructions strictly throughout the procedure.

3. Appropriate protective measures must be taken, including wearing a lab coat and latex gloves.

4. If the concentration of the substance falls outside the detection range, perform an additional dilution or concentration step on the sample.

5. It is recommended to perform a pre-test if your sample type is not listed in the instruction manual.

6. Experimental results are closely related to reagent quality, operator technique, environmental conditions, and other factors. Assay Genie guarantees the quality of the kits only and is NOT responsible for sample consumption resulting from use of the assay kits. It is advisable to estimate the expected sample usage and reserve sufficient samples before starting the experiment.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.