

Version 4b, Last updated 12 March 2026

# ab273315 Acyl-CoA Synthetase Assay Kit (Fluorometric)

View Kit datasheet: <https://www.abcam.com/ab273315>  
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<https://www.abcam.co.jp/ab273315> for Japan)

For the determination of ACS activity in various cells and tissues

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

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## 1. Overview

Acyl-CoA Synthetase (ACS) Assay Kit (Fluorometric) (ab273315) 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.

## 2. Protocol Summary

Prepare samples as directed



Prepare all reagents as directed



Prepare standard curve and measure fluorescence at Ex/Em = 535/587 nm. Calculate slope



Add Positive Control, Samples, Background Control to appropriate wells and adjust volume to 50  $\mu$ L



Add Reaction Mix or Background Mix (50  $\mu$ L)



Measure fluorescence (Ex/Em= 535/587 nm) in a kinetic mode for 30 min at 37°C.

### 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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- If applicable, please refer to the current Safety Data Sheet (SDS) provided with this product for safety, handling, and disposal information. The most up to date and current versions are available on our website <https://www.abcam.com/en-us>.

### 4. Storage and Stability

**Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 2 months from receipt.**

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. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

## 6. Materials Supplied

Item	Quantity	Storage temperature (before prep)
Assay Buffer 5	25 mL	-20°C
ACS Substrate	1 mL	-20°C
Acyl CoA Enzyme Mix	1 vial	-20°C
ACS Converter	1 vial	-20°C
ACS Developer	1 vial	-20°C
ACS Positive Control	1 vial	-20°C
OxiRed™ Probe	0.2 mL	-20°C
H <sub>2</sub> O <sub>2</sub> Standard	100 µL	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and ACS Assay Buffer, and OxiRed™ Probe as OxiRed Probe and ACS Probe. The composition has not changed.

## 7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Fluorescence microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96-well black plate with flat bottom

## 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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- 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.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

## 9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

### 9.1 **Assay Buffer 5:**

Store at -20 °C. Bring to room temperature before use.

Use within two months.

### 9.2 **ACS Substrate:**

Ready to use. Thaw and warm to room temperature.

### 9.3 **Acyl CoA Enzyme Mix:**

Reconstitute with 220 µl Assay Buffer 5, pipette up and down to mix.

Store at -20°C, protected from light. Use within two months.

### 9.4 **ACS Converter:**

Reconstitute with 220 µl dH<sub>2</sub>O. Aliquot and store at -20°C.

Use within two months.

### 9.5 **ACS Developer:**

Reconstitute with 220 µl dH<sub>2</sub>O. Aliquot and store at -20°C.

Use within two months.

### 9.6 **ACS Positive Control:**

Reconstitute with 100 µl Assay Buffer 5, pipette up and down to mix.

Aliquot and store at -20°C, protected from light.

Use within two months.

### 9.7 **OxiRed™ Probe:**

Ready to use. Thaw and warm to room temperature.

### 9.8 **H<sub>2</sub>O<sub>2</sub> Standard:**

Ready to use. Thaw and warm to room temperature.

## 10. Sample Preparation

### 10.1 Tissue/cell lysate preparation:

10.1.1 Homogenize tissue (10 mg) or cells ( $1 \times 10^6$ ) with 100  $\mu$ l ice cold Assay Buffer 5.

10.1.2 Centrifuge at 10,000x g and 4°C for 15 min.

10.1.3 Transfer the supernatant to a fresh tube.

**Δ Note:** 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.

**Δ Note: 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.

## 11. Standard Curve

11.1 Prepare a 10 mM solution of H<sub>2</sub>O<sub>2</sub> Standard by diluting 10  $\mu$ l H<sub>2</sub>O<sub>2</sub> Standard with 870  $\mu$ l dH<sub>2</sub>O.

11.2 Prepare a 0.1 mM solution of H<sub>2</sub>O<sub>2</sub> Standard by diluting 10  $\mu$ l of the 10 mM H<sub>2</sub>O<sub>2</sub> Standard into 990  $\mu$ l.

11.3 Add 0, 2, 4, 6, 8, and 10  $\mu$ l of the 0.1 mM H<sub>2</sub>O<sub>2</sub> Standard into a 96-well plate and adjust the final volume to 50  $\mu$ l/well with Assay Buffer 5. This will generate 0, 0.2, 0.4, 0.6, 0.8, 1.0 nmol/well H<sub>2</sub>O<sub>2</sub> Standard respectively.

Standard #	0.1 mM H <sub>2</sub> O <sub>2</sub> - Standard ( $\mu$ L)	Assay Buffer 5 ( $\mu$ L)	H <sub>2</sub> O <sub>2</sub> (nmol/well)
1	0	50	0
2	2	48	0.2
3	4	46	0.4
4	6	44	0.6
5	8	42	0.8
6	10	40	1.0

## 12. Assay Procedure

Thaw all reagents thoroughly and mix gently.

- 12.1 Add the same desired volume (2-20  $\mu\text{l}$ ) of the Sample supernatant to both the wells (Sample & Sample Background).
- 12.2 Positive Control: Add 2-4  $\mu\text{l}$  Positive Control to Positive Control well(s).
- 12.3 Adjust the volume to 50  $\mu\text{l}$ /well with Assay Buffer 5. Mix well.

### Reaction Mix:

- 12.3.1 Prepare enough reagents for the number of assays to be performed. For each well, prepare 50  $\mu\text{l}$  of each Mix:

Component	Reaction Mix ( $\mu\text{L}$ )	Background Control Mix ( $\mu\text{L}$ )
Assay Buffer 5	41.6	43.6
ACS Substrate	2	0
Acyl CoA Enzyme Mix	2	2
ACS Converter	2	2
ACS Developer	2	2
OxiRed™ Probe	0.4	0.4

- 12.3.2 Add 50  $\mu\text{l}$  of the Reaction Mix into Standard Positive control and Sample wells.

- 12.3.3 Add 50  $\mu\text{l}$  of the Background Control Mix into Sample Background well(s).

**Δ Note:** The Sample Background reading should be subtracted from all Sample reading.

- 12.3.4 Measure fluorescence (Ex/Em= 535/587 nm) in a kinetic mode for 30 min at 37°C.

- 12.3.5 The Standard Curve should reach the endpoint (maximal signal) within 10 min.

**Δ 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 (t1 and t2) 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.

### 13. Calculations

- 13.1 Subtract the 0 nmol/well Standard reading from all Standard readings.
- 13.2 Plot the H<sub>2</sub>O<sub>2</sub> Standard Curve.
- 13.3 Subtract the Sample Background reading from all Sample readings within t<sub>1</sub> and t<sub>2</sub>.
- 13.4 Apply the  $\Delta\text{RFU} = \text{RFU}_1 - \text{RFU}_2$  to the Standard Curve to get B nmol of H<sub>2</sub>O<sub>2</sub> generated during the reaction time ( $\Delta t = t_2 - t_1$ ).
- 13.5 Sample Acyl-CoA Synthetase Activity is calculated as:

$$\text{Sample ACS activity} = \frac{B}{(\Delta t \times V)} \times D \text{ (nmol/min/ml)}$$

B = H<sub>2</sub>O<sub>2</sub> amount from the Standard Curve

$\Delta t$  = Reaction time (min)

V = Sample volume added into the reaction well (ml)

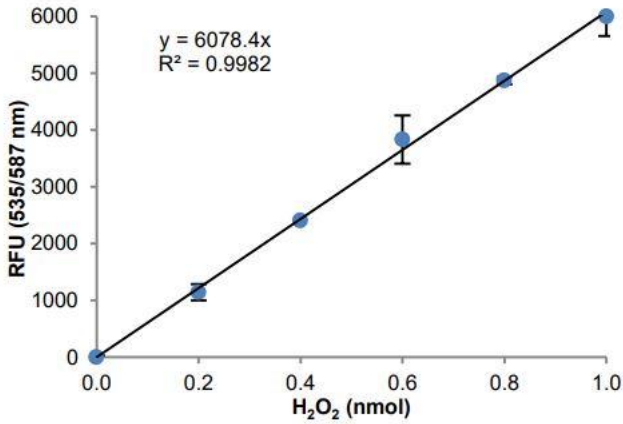
D = Dilution factor (D = 1 for undiluted Samples)

#### Unit definition:

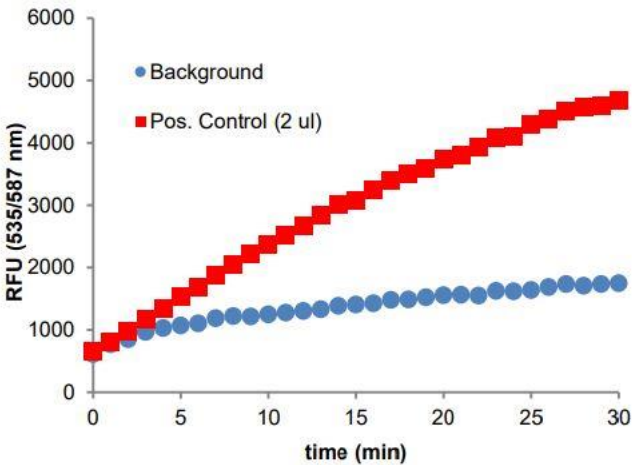
One unit of Acyl-CoA Synthetase is the amount of enzyme that generates 1.0  $\mu\text{mole}$  of Umbelliferone per min at pH 7.5 at 37°C.

## 14. Typical Data

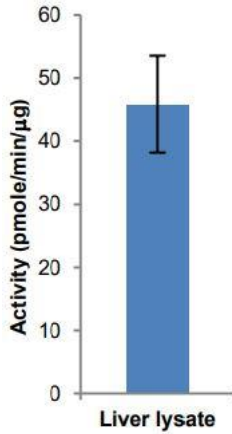
**Typical standard curve** – data provided **for demonstration purposes only**. A new standard curve must be generated for each assay performed.



**Figure 1.** H<sub>2</sub>O<sub>2</sub> Standard Curve.



**Figure 2.** Reaction kinetics of ACS Positive Control.



**Figure 3.** 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.

## 15. FAQ / Troubleshooting

General troubleshooting points are found at

<https://www.abcam.com/en-us/products/biochemical-assays>.

## 16. Notes

## Technical Support

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