

ACYL-CoA SYNTHETASE [ACS]

from *Pseudomonas fragi*
(Acid: CoA ligase (AMP forming), EC 6.2.1.3)



Preparation and Specification

Appearance : White amorphous powder, lyophilized
Specific activity : More than 2 U/mg solid

Properties

Substrate specificity	: See Table 1	
Molecular weight	: 60 kDa (Sephadex G-150) , 62 kDa (SDS-PAGE)	
Isoelectric point	: pH 5.2	
Michaelis constants	: Palmitic acid $1.1 \times 10^{-5}\text{M}$ ATP $1.7 \times 10^{-4}\text{M}$ CoA $3.2 \times 10^{-4}\text{M}$	
Optimum pH	: Palmitic acid 7.7 Serum fatty acids 7.7	Figure 1 Figure 2
pH stability	: 6.0–8.0 (37°C, 2 hr)	Figure 3
Thermal stability	: Stable at 50°C and below (pH 7.5, 10 min)	Figure 4
Storage stability	: At least one year at –20°C	Figure 5
Effect of various chemicals	: See Table 2 and Table 3	
Stabilizer	: ATP	
Activator	: Triton X-100	

Applications for Diagnostic Test

This enzyme is useful for enzymatic determination of **fatty acid** when coupled with Acyl-CoA oxidase (T-17) .

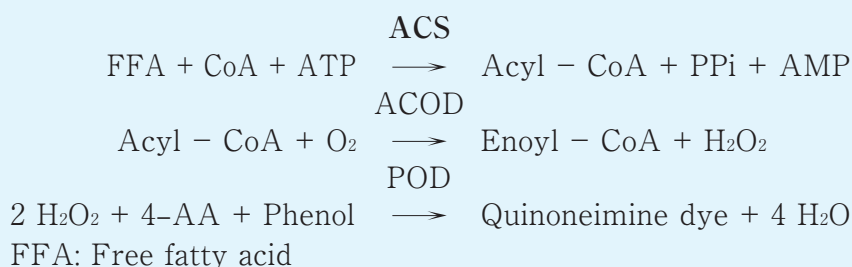


Table 1. Substrate specificity (Fatty acids)

Substrate	Relative activity (%)	Km value (10^{-5} M)
Caproic acid (6:0)	38	
Caprylic acid (8:0)	64	3.7
Capric acid (10:0)	24	0.5
Lauric acid (12:0)	21	0.95
Myristic acid (14:0)	40	0.71
Palmitic acid (16:0)	66	1.10
Stearic acid (18:0)	78	3.0
Arachidic acid (20:0)	100	
Myristoleic acid (14:1)	35	
Palmitoleic acid (16:1)	40	
Palmitelaidic acid (16:1)	48	
Oleic acid (18:1)	78	0.91
Elaidic acid (18:1)	60	
Linoleic acid (18:2)	57	0.34
Linolenic acid (18:3)	62	1.10
Arachidonic acid (20:4)	63	
Erucic acid (22:1)	92	
Nervonic acid (24:1)	9	

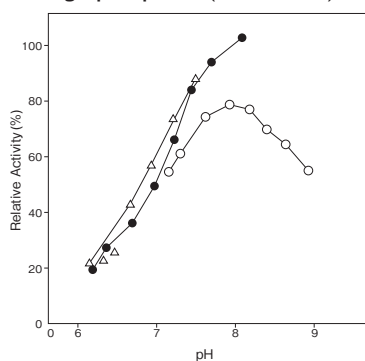
Table 3. Effect of metal ions on ACS activity

Metal ion	Concentration	Relative activity (%)
None	–	38
KCl	0.1M	29
NaCl	0.1M	35
LiCl	0.1M	26
NH ₄ Cl	0.1M	29
MgCl ₂	1mM	100
CaCl ₂	1mM	94
ZnCl ₂	1mM	31
BaCl ₂	1mM	38
MnCl ₂	1mM	117
CuCl ₂	1mM	0
NiCl ₂	1mM	87
EDTA	1mM	0
1mM MgCl ₂ + CaCl ₂	1mM	103
1mM MgCl ₂ + ZnCl ₂	1mM	55
1mM MgCl ₂ + CuCl ₂	1mM	0
1mM MgCl ₂ + NiCl ₂	1mM	99
1mM MgCl ₂ + BaCl ₂	1mM	100
1mM MgCl ₂ + MnCl ₂	1mM	117

Table 2. Effect of detergents on ACS activity

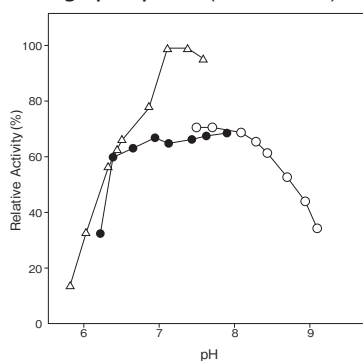
Detergent (%)	Relative activity (%)
None	100
Deoxycholate 0.1	69.6
0.25	41.1
SDS 0.1	0
Cetyltrimethyl-ammoniumchloride 0.1	96.4
0.25	0
Cetylpyridinium chloride 0.1	96.4
0.25	0
Sarcosinate PN 0.1	64.3
0.25	0

Fig.1 pH Optimum (Palmitic acid)



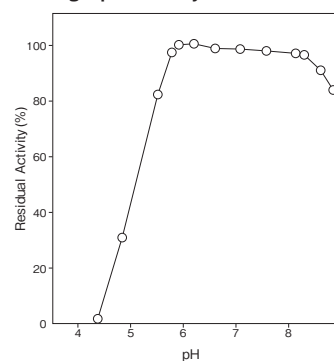
△ : 3,3-Dimethylglutarate-NaOH buffer
 ● : Phosphate buffer
 ○ : Tris-HCl buffer

Fig.2 pH Optimum (Serum NEFA)



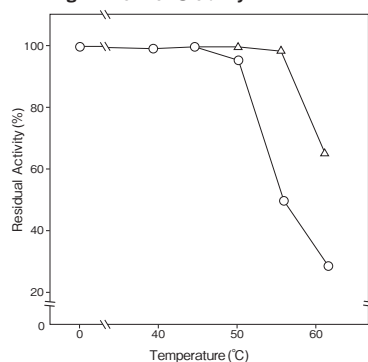
△ : 3,3-Dimethylglutarate-NaOH buffer
 ● : Phosphate buffer
 ○ : Tris-HCl buffer

Fig.3 pH Stability



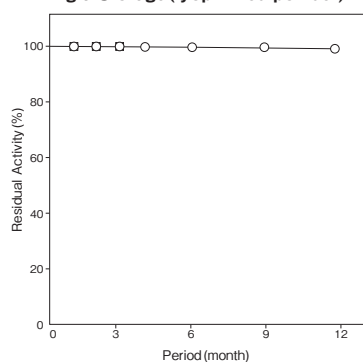
37°C, 2 hr
 pH 4.0–6.5 3,3-Dimethylglutarate-NaOH buffer
 pH 6.5–7.5 Phosphate buffer
 pH 7.5–9.0 Tris-HCl buffer

Fig.4 Thermal Stability



pH 7.5, 10 min.
Phosphate buffer
△: +2 mM ATP
○: Non addition

Fig.5 Storage (lyophilized powder)

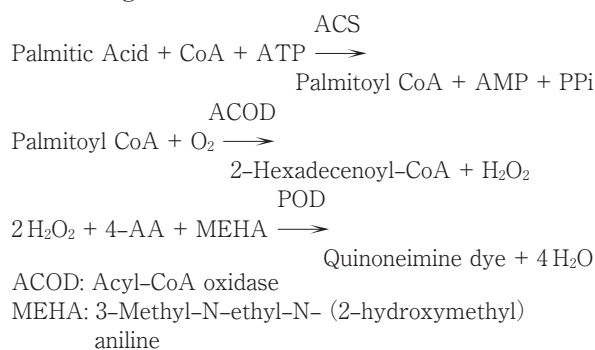


○: -20°C
□: 5°C

Assay

Principle

The assay is based on the increase in absorbance at 550 nm as the formation of quinoneimine dye proceeds in the following reactions:



Unit definition

One unit is defined as the amount of enzyme which converts 1 μ mole of fatty acid to acyl-CoA per minute at 37°C under the conditions specified in the assay procedure.

Reagents

1. Reaction mixture for the first reaction

0.2 M KH_2PO_4 - K_2HPO_4 buffer pH 7.5	0.20 ml
10 mM ATP solution pH 7.5	0.10 ml
10 mM MgCl_2 solution	0.10 ml
1 mM Palmitic acid-5 % (W/V)	
Triton X-100 solution pH 7.5 ¹⁾	0.20 ml
Distilled water	0.35 ml
10 mM CoA solution pH 6.5 ²⁾	0.05 ml

1): 1 mM Palmitic acid-5 % (W/V) Triton X-100 solution pH 7.5
Dissolve 26 mg of palmitic acid with 90 ml of 5% (W/V) Triton X-100, adjust pH to 7.5 at 25°C with 4 N NaOH, add 5% (W/V) Triton X-100 to make a total of 100 ml.

2): 10mM CoA solution pH 6.5
Dissolve 154 mg (purity calculation) of CoA with 15 ml of distilled water, adjust pH to 6.5 at 25°C with 4 N NaOH, and add distilled water to make a total of 20 ml.

2. Reaction mixture for the second reaction

0.2 M KH_2PO_4 - K_2HPO_4 buffer pH 7.5	0.50 ml
20mM NEM	0.10 ml
15mM 4-AA solution	0.30 ml
0.3% (W/V) MEHA solution pH 5.8	0.25 ml
100 U/ml POD solution ³⁾	0.10 ml
0.5% (W/V) NaN_3 solution	0.10 ml
Distilled water	0.55 ml
120 U/ml ACOD solution ⁴⁾	0.10 ml

NEM: N-Ethylmaleimide

3): 100 U/ml POD solution

Dissolve 1,000 U (PPU) of POD with 10 ml of distilled water.

4): 120 U/ml ACOD solution

Dissolve 1,200 U of ACOD with 10 ml of ACOD dilution buffer ^{※)}.

※): ACOD dilution buffer

Dissolve 1.36 g of KH_2PO_4 and 1.82 g of ATP with distilled water, adjust pH to 7.0 with 4 N NaOH, add 10 ml of 1 mM FAD, and finally add distilled water to make a total of 1 L.

3. Enzyme dilution buffer

10 mM KH_2PO_4 - K_2HPO_4 buffer (pH 7.5) containing 2 mM ATP, 0.5%(W/V) BSA and 0.1 %(W/V) Triton X-100.

4. Reagents:

ATP ($2\text{Na} \cdot 3\text{H}_2\text{O}$): Kyowa Hakko Co., Ltd.

CoA (Coenzyme A): KOHJIN

Palmitic acid :

FUJIFILM Wako Pure Chemical Corporation #169-00105

Triton X-100: The Dow Chemical company

NEM: FUJIFILM Wako Pure Chemical Corporation

Special grade #058-02061

4-AA: NACALAI TESQUE, INC.

Special grade #01907-52

MEHA: Tokyo Kasai Kogyo Co., Ltd. #E0220

POD: Sigma Chemical Co. Type II #P-8250

ACOD: Nagase Diagnostics Co., Ltd. #T-17

FAD (2Na): Kyowa Hakko Co., Ltd.

BSA: Millipore Fraction V pH 5.2 #81-053

ATP: Adenosine triphosphate
FAD: Flavine adenine dinucleotide

■ Enzyme solution

Weigh about 20 mg of test sample exactly and add enzyme dilution buffer to make a total of 20 ml. Dissolve it with enzyme dilution buffer to adjust the concentration to within 0.04–0.06 U/ml.

■ Procedure

1. Pipette accurately 1.0 ml of reagent mixture for the first reaction into a small test tube and preincubate at 37°C.
2. After 5 min, add exactly 50 μ l of enzyme solution and mix to start the first reaction at 37°C.
- ※ In the case of a test blank, add 50 μ l of enzyme dilution buffer in place of enzyme solution.
3. After 10 min, add 2.0 ml of reagent mixture for the second reaction to stop the first reaction and mix to start the second reaction at 37°C.
4. After 5 min, measure the absorbance at 550 nm.

$$\begin{aligned} \text{Absorbance sample} &: A_s \\ \text{blank} &: A_b \\ \Delta A &= (A_s - A_b) \leq 0.30 \text{ Abs} \end{aligned}$$

■ Calculation

$$\text{Activity (U/mg)} = \frac{\Delta A/10}{32.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

32.0 : millimolar extinction coefficient of quinoneimine dye at 550 nm ($\text{cm}^2 / \mu\text{mole}$)

1/2 : a multiplier derived from the fact that 2 mole of H_2O_2 produces 1 mole of quinoneimine dye

10 : reaction time (min)

3.05 : final volume (ml)

0.05 : volume of enzyme solution (ml)

X : concentration of ACS in enzyme solution (mg/ml)

Storage

Storage at -20°C in the presence of a desiccant is recommended. Enzyme activity will be retained for at least one year under this condition.

References

1. Yamada, H., Shimizu, S. and Tani, Y. (1980) Vitamin (Japanese), **54**, 489.
2. Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1980) Anal. Biochem., **107**, 193.
3. Okabe, H., Uji, Y., Nagashima, K. and Noma, A. (1980) Clin. Chem., **26**, 1540.
4. Shimizu, S., Inoue, K., Tani, Y. and Yamada, H. (1979) Anal. Biochem., **98**, 341.

ACS 活性測定法 (Japanese)

I. 試薬液

1. 第一反応試薬混合液

0.2M KH_2PO_4 - K_2HPO_4 緩衝液 pH7.5	0.20 ml
10mM ATP 溶液 pH7.5	0.10 ml
10mM 塩化マグネシウム溶液	0.10 ml
1mM パルミチン酸 -5% (W/V)	
トリトン X-100 溶液 pH7.5 ¹⁾	0.20 ml
精製水	0.35 ml
10mM CoA 溶液 pH6.5 ²⁾	0.05 ml

1): 1mM パルミチン酸 -5% (W/V) トリトン X-100 溶液 pH7.5
 パルミチン酸 26mg を 5% (W/V) トリトン X-100 溶液 90ml で加温溶解した後、4N NaOH で pH7.5 (25°C) に調整し、5% (W/V) トリトン X-100 溶液で全容 100ml とする。

2): 10mM CoA 溶液 pH6.5
 CoA 154mg (純度換算) を精製水 15ml に溶解した後、4N NaOH で pH6.5 (25°C) に調整し、精製水で全容 20ml とする。
2. 第二反応試薬混合液

0.2M KH_2PO_4 - K_2HPO_4 緩衝液 pH7.5	0.50 ml
20mM NEM 溶液	0.10 ml
15mM 4-AA 溶液	0.30 ml
0.3% (W/V) MEHA 溶液 pH5.8	0.25 ml
100U/ml POD 溶液 ³⁾	0.10 ml

0.5% (W/V) NaN_3 溶液	0.10 ml
精製水	0.55 ml
120U/ml ACOD 溶液 ⁴⁾	0.10 ml

3): 100U/ml POD 溶液

POD 1,000 単位 (PPU) を精製水 10ml で溶解する。

4): 120U/ml ACOD 溶液

ACOD 1,200 単位 (U) を ACOD 希釈緩衝液 ^{※)} 10ml で溶解する。

※): ACOD 希釈緩衝液

KH_2PO_4 1.36g と ATP 1.82g を精製水に溶解した後、4N NaOH で pH7.0 (25°C) に調整し、さらに 1mM FAD 溶液 10ml を加えて精製水で全容 1L とする。

3. 酵素溶解希釈用液

2mM ATP と 0.5% (W/V) BSA 及び 0.1% (W/V) トリトン X-100 を含む 10mM KH_2PO_4 - K_2HPO_4 緩衝液 pH7.5

4. 試薬

ATP (アデノシン三リン酸・ $2\text{Na} \cdot 3\text{H}_2\text{O}$):

協和発酵製

CoA (コエンザイム A): 興人製

パルミチン酸: 富士フィルム和光純薬製 特級

#169-00105

トリトン (トリトン X-100): Dow Chemical 製

NEM (N-エチルマレイミド):

富士フィルム和光純薬製 特級 #058-02061

4-AA: ナカライトスク製 特級 #01907-52

MEHA [N-エチル-N-2-ヒドロキシエチル-m-トルイジン]:

東京化成製 #E0220

POD: シグマ製 Type II #P-8250

ACOD (アシル-CoA 酸化酵素):

ナガセダイアグノスティックス製 #T-17

FAD (フラビンアデニンジヌクレオチド・2Na):

協和発酵製

BSA: Millipore 製 Fraction V pH5.2 #81-053

II. 酵素試料液

検品約 20mg を精密に量り、酵素溶解希釈用液に溶解して全容 20ml とする。

その液を酵素溶解希釈用液で 0.04~0.06U/ml 濃度となるように適宜希釈する。

III. 測定操作法

1. 小試験管に第一反応試薬混合液 1.0ml を正確に分注して 37℃ で予備加温する。
2. 5 分経過後、酵素試料液 50 μ l を加えて混和し、37℃ で反応を開始する。

※盲検は酵素試料液の代わりに酵素溶解希釈用液 50 μ l を加える。

3. 10 分経過後、第二反応試薬混合液 2.0ml を正確に加えて混和し、第一反応を停止させ、37℃ で第二反応を開始する。

4. 5 分経過後、550nm における吸光度を測定する。求められた吸光度を試料液は As、盲検液は Ab とする。

$$\Delta A = (A_s - A_b) \leq 0.30 \text{ Abs}$$

IV. 計算

$$\text{活性 (U/mg)} = \frac{\Delta A / 10}{32.0 \times 1/2} \times \frac{3.05}{0.05} \times \frac{1}{X}$$

32.0: キノノイミン色素の 550nm におけるミリモル分子吸光係数 ($\text{cm}^2 / \mu\text{mole}$)

1/2: H_2O_2 2 モルからキノノイミン色素 1 モルが生成することによる係数

10 : 反応時間 (min)

3.05: 反応総液量 (ml)

0.05: 反応に供した酵素試料液量 (ml)

X : 酵素試料液中の検品濃度 (mg/ml)