

EZ- Free Fatty Acid Assay Kit

Metabolism assay kit
(Colorimetric/Fluorometric)

Cat. No. DG-FFA100

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

▪ Product Description

Fatty Acid plays a very important role in normal metabolism and many diseases. They are precursors of many bioactive classes of compounds such as prostaglandins, leucotrienes, etc. and have been implicated in various functions such as autism, immune system and inflammatory response.

EZ-Free Fatty Acid Assay Kit is a product that can detect fatty acids in various biological samples such as serum, plasma and other body fluids, food, and growth media using a convenient and highly sensitive enzyme-based method. It uses the principle that fatty acids present in the sample are converted to CoA derivatives and then oxidized to generate an intermediate (H_2O_2) that induces color development in the probe. Absorbance (OD 570 nm) and fluorescence (Ex/Em = 535/595 nm) can be easily measured.

▪ Contents and Storage Conditions

Component	100 assay	Cap Cord	Storage
Fatty Acid Assay buffer	25 mL	-	-20°C
Fatty Acid Enzyme mix (Lyophilized)	1 vial	Red	-20°C
ACS Reagent (Lyophilized)	1 vial	Blue	-20°C
Cofactor mixture (Lyophilized)	1 vial	Orange	-20°C
Enhancer	200 µL	Silver	-20°C
Fatty Acid Probe	200 µL	Yellow	-20°C
Fatty Acid Standard (1 mM)	300 µL	Green	-20°C

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

* In terms of the number of tests that can be performed with this product, 100 assays means that it provides reagents that can process 100 wells based on 1 well of a 96 well plate. Among these, considering standard, blank, duplication processing per sample, etc., the actual number of samples that can be tested is in the range of 20 to 40 samples. Review the product instructions carefully and determine the number of kits required considering the characteristics of the sample you wish to test.

▪ Preparation of Reagent

Component	Preparation	Storage and Stability
Enhancer Fatty Acid Probe	Use after sufficiently dissolving at room temperature.	Store the remaining solution at -20°C and use it within 1 month.
ACS Reagent Cofactor mixture Fatty Acid Enzyme mix (Lyophilized)	Add 220 μl Assay Buffer and mix well using a pipette.	The mixed solution is stable for 1 months at -20°C.
Fatty Acid Standard	Sufficiently dissolve at room temperature and incubate in an 80°C water bath for 1 minute or in a heat block for 3 minutes. (<u>Make sure the cap is closed properly</u>) Use after sufficiently cooling down to room temperature.	Store the remaining solution at -20°C and use it within 1 month.

* Assay buffer is used after sufficiently warming up to room temperature before experiment.

* When using a cold buffer, enzyme activity may be inhibited, affecting measurement results.

▪ General Protocol

1. Sample preparation

After adding 2-50 μl of the prepared sample into a 96 well plate, adjust the final volume to 50 μl with Assay buffer. (n \geq 2)

1) Liquid Samples (plasma, serum, urine and other biological fluids)

: You can use it for analysis right away.

2) Cell or Tissue

① Prepare 1 x 10⁶ cells or 10 mg tissue sample.

② PBS를 이용하여 Cell 또는 tissue sample을 washing 해줍니다.

③ Add 200 μl of chloroform-Triton X-100(1 % Triton X-100 in pure chloroform) to the cell or

tissue sample and homogenize on ice.

- ④ Centrifuge at maximum speed for 5-10 minutes.
- ⑤ Take the organic phase (lower phase), dry at 50°C to remove chloroform, and then completely remove remaining chloroform in a vacuum dryer for 30 minutes..
- ⑥ Add 200 μl of free fatty acid assay buffer to the dried lipids and vortex for 5 minutes.

* The solution may be cloudy or milky, but this does not affect the analysis.

* When testing a larger amount of sample, the reagent at each step can be increased proportionally to match the sample amount.

3) For unknown samples or samples being measured for the first time, it is recommended to use the product after conducting a preliminary experiment to ensure that the measured values are within the standard curve.

4) If the measured value of a sample has a high background value, prepare the same amount of sample used for measurement as a background control.

2. Standard preparation

1) Colorimetric method

Add 0, 2, 4, 6, 8, and 10 μl of 1 mM Fatty Acid Standard into a 96 well plate and adjust the final volume to 50 μl with assay buffer. Then, 0, 2, 4, 6, 8, and 10 are added to each plate. A standard set of nmol/well is created.

Standard No.	Volume of 1mM Fatty Acid Standard	Volume of Assay buffer	Final standard volume in well	Final standard Fatty Acid Conc. (nmol/well)
1	0 μl	50 μl	50 μl	0
2	2 μl	48 μl	50 μl	2
3	4 μl	46 μl	50 μl	4
4	6 μl	44 μl	50 μl	6
5	8 μl	42 μl	50 μl	8
6	10 μl	40 μl	50 μl	10

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

2) Fluorometric method

Prepare 0.1 mM Fatty Acid standard solution by mixing 10 μl of 1 mM Fatty Acid standard solution and 90 μl of assay buffer, and add 0, 2, 4, 6, 8, and 10 μl into each

well. By adjusting the final volume to 50 μl with assay buffer, a standard set of 0, 0.2, 0.4, 0.6, 0.8, and 1.0 nmol/well is added in each plate.

Standard No.	Volume of 0.1mM Fatty Acid Standard	Volume of Assay buffer	Final standard volume in well	Final standard Fatty Acid Conc. (nmol/well)
1	0 μl	50 μl	50 μl	0
2	2 μl	48 μl	50 μl	0.2
3	4 μl	46 μl	50 μl	0.4
4	6 μl	44 μl	50 μl	0.6
5	8 μl	42 μl	50 μl	0.8
6	10 μl	40 μl	50 μl	1.0

* For accurate measurement, it is recommended to prepare and conduct experiments with more than two replicates each of standards and samples.

* Standard recommends measurement during each experiment.

3. ACS (acyl-CoA Synthesis) Reagent / Cofactor mixture

Add 2 μl of ACS Reagent and Cofactor mixture to each standard and sample well, block light, and incubate at 37°C for 30 minutes.

4. Reaction mixture preparation

This is the volume based on one well assay. Calculate the assay volume to be used in the experiment and prepare the reaction mix considering the loss volume.

1) Colorimetric method

Components	Reaction mixture
Fatty Acid Assay buffer	44 μl
Fatty Acid Enzyme mix	2 μl
Enhancer	2 μl
Fatty Acid Probe	2 μl
Total	50 μl

2) Fluorometric method

Components	Reaction mixture
Fatty Acid Enzyme mix	45.4 μl
Enhancer	4.6 μl
Fatty Acid Assay buffer	50 μl
Total	50 μl

5. Add 50 μl of the reaction mixture to each well containing the ATP standard and sample using a multi pipette, then mix well.

6. After incubation the plate at room temperature blocked from light for 30 minutes, shake gently and measure using a microplate reader.

1) Colorimetric : 570 nm

2) Fluorometric: (Excitation/Emission): 535 nm / 595 nm

▪ Calculation

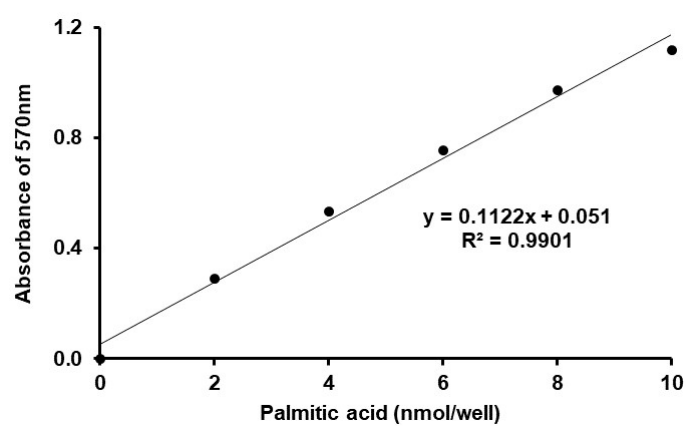
1. Standard 1 value (blank) is subtracted from all measured values.
2. Calculate the average value of duplicate measurements of each standard well and sample well.
3. Determine a standard curve using Fatty Acid standard absorbance.
4. Calculate the amount of Fatty Acid in the sample by substituting the sample measurement value into the standard curve.
5. Based on the amount of Fatty Acid in the sample calculated in 4, use the following equation, calculate the concentration of fatty acid in the sample.

$$\text{Fatty acid concentration in the sample (C)} = B/V \times D \text{ (nmol}/\mu\text{l} \text{ or mM)}$$

B : Amount of fatty acid (nmol) in the measurement well obtained from the standard curve

V : Amount of sample added into the well (μl)

D : Sample dilution factor (ex. If diluted 2 times, it is calculated as x2, not x1/2)



Fatty acid standard curve. Assay was performed following the kit protocol.

▪ Related Product

	Products	Catalog No.	Assay
Oxidative Stress Assay Kit	EZ-Superoxide Dismutase (SOD) Assay Kit (Colorimetric)	DG-SOD400	400 Assay
	EZ-Glutathione Assay Kit (Colorimetric)	DG-GLU200	200 Assay
	EZ-Catalase Assay Kit (Fluorometric/Colorimetric)	DG-CAT400	400 Assay
	EZ-Hydrogen peroxide/Peroxidase Assay Kit (Fluorometric/Colorimetric)	DG-PER500	500 Assay
	EZ-Lipid Peroxidation (TBARS) Assay Kit (Colorimetric)	DG-TBA200	200 Assay
	EZ-Total Antioxidant Capacity (TAC) Assay Kit (Colorimetric)	DG-TAC200	200 Assay
	EZ-DPPH Antioxidant Assay Kit (Colorimetric)	DG-DPH400	400 Assay
	EZ-ABTS Antioxidant Assay Kit (Colorimetric)	DG-ABT400	400 Assay
	EZ-Glutathione Peroxidase Assay Kit (Colorimetric)	DG-GPX100	100 Assay
Metabolism Assay Kit	EZ-Lactate Assay Kit (Colorimetric)	DG-LAC100	100 Assay
	EZ-Acetylcholinesterase Assay Kit (Colorimetric)	DG-ACE100	100 Assay
	EZ-Ascorbic Acid Assay Kit (Colorimetric)	DG-ASC100	100 Assay
	EZ-ATP Assay Kit (Fluorometric/Colorimetric)	DG-ATP100	100 Assay
	EZ-Free Fatty Acid Assay Kit (Fluorometric/Colorimetric)	DG-FFA100	100 Assay
	EZ-Free Glycerol Assay Kit (Fluorometric/Colorimetric)	DG-FGC100	100 Assay
	EZ-Glucose Assay Kit (Fluorometric/Colorimetric)	DG-GCS100	100 Assay
	EZ-HDL, LDL/VLDL Assay Kit (Fluorometric/Colorimetric)	DG-CHO100	100 Assay
	EZ-Total Cholesterol Assay Kit (Fluorometric/Colorimetric)	DG-TSC100	100 Assay
	EZ-Triglyceride Quantification Assay Kit (Fluorometric/Colorimetric)	DG-TGC100	100 Assay
	EZ-Nitric Oxide Assay kit (Colorimetric)	DG-NO500	500 Assay
	EZ-Total Collagen Assay Kit (Colorimetric)	DG-COL100	100 Assay
	EZ-Ethanol Assay Kit (Colorimetric)	DG-ETH100	100 Assay