Signosis
BioSignal Capture

Free Fatty Acid Assay Kit

Catalog Number EA-7012 **(For Research Use Only)**

Introduction

The Free Fatty Acid (FFA) assay kit utilizes a series of enzyme reactions to measure free fatty acid levels in samples. First, Acyl-Coa synthetase attaches coenzyme A to the FFAs to form Acyl-Coa. Then, Acyl-Coa oxidase reacts with Acyl-Coa to produce hydrogen peroxide. The FFA level in the sample is determined by quantifying the hydrogen peroxide generated by the enzyme reaction with a fluorogenic probe that can be measured with a spectrophotometer.

Materials provided with the kit

- KRPG buffer (RT)
- FFA Standard (-20ºC)
- Acyl-Coa synthetase (ACS) Reagent (-20ºC)
- Acyl-Coa oxidase (ACO) Reagent (-20ºC)
- H2O2 Probe (-20ºC)
- HRP Reagent (4ºC)
- Aluminum plate seal (RT)

Material required but not provided

- 96-well clear microplate
- Microplate reader capable of measuring absorbance at 560 nm

Plasma Sample Preparation

1. Centrifuge citrated or EDTA-collected blood at 4°C (1,000 x *g* for 10 minutes) to separate plasma from erythrocytes. Alternatively, blood collected without anticoagulant can be centrifuged to collect serum.
2. Transfer the plasma layer to a new tube without disturbing the buffy layer.
3. The plasma may be assayed directly or stored away at -80°C.

Cell Sample Preparation

1. From a 96-well culture plate, detach adherent cells with trypsin. For suspension cells, centrifuge at 1,000 x g for 5 minutes to pellet cells.
2. Wash the cells twice with cold PBS to remove residual media.
3. Resuspend the cells in 1 mL of PBS and homogenize using a tissue grinder or sonicator.
4. Add 2 mL of chloroform and 1 mL of methanol to the homogenized cell sample and mix thoroughly by vortexing for 30 seconds.
5. Add 0.5 mL of ddH_2O to the mixture and vortex again for 30 seconds to induce phase separation.
6. Centrifuge the sample at 1,500 x g for 10 minutes at room temperature to separate the phases.
7. Carefully collect the lower chloroform phase containing the lipids and transfer to a new tube.
8. Vacuum dry the lipid sample until all of the chloroform is evaporated.
9. Reconstitute the dry lipid sample in PBS.
10. The lipid sample may be assayed directly or stored at -80°C.
11. Dilute the lysates 1:10 with KRPG buffer before beginning assay.

Tissue Sample Preparation

1. Weigh 100 mg of tissue and place in a tube.
2. Add 1 mL of cold methanol and homogenize using a tissue grinder.
3. Add 2 mL of chloroform to the homogenized tissue sample and mix thoroughly by vortexing for 30 seconds.
4. Add 0.5 mL of ddH_2O to the mixture and vortex again for 30 seconds to induce phase separation.
5. Centrifuge the sample at 1,500 x g for 10 minutes at room temperature to separate the phases.
6. Carefully collect the lower chloroform phase containing the lipids and transfer to a new tube.
7. Vacuum dry the lipid sample until all of the chloroform is evaporated.
8. Reconstitute the dry lipid sample in PBS.
9. The lipid sample may be assayed directly or stored at -80°C.
10. Dilute the lysates 1:10 with KRPG buffer before beginning assay.

FFA Measurement

1. Using the provided 1 mM FFA standard, prepare a standard curve dilution in a 96-well clear plate as described in the table below:

Standard #	1	2	3	4	5	6
FFA Standard Volume (µL)	12.5	10	7.5	5	2.5	0
KRPG Buffer (µL)	37.5	40	42.5	45	47.5	50
FFA Final Concentration (µM)	250	200	150	100	50	0

2. Add 50 µL of plasma or serum samples to each well of the plate. Dilute samples in KRPG buffer if necessary.
3. Add 5 µL of ACS Reagent to each well.
4. Mix the samples and incubate the plate at 37°C for 30 minutes.
5. Prepare Reaction master mix (50uL per well) according to the table below:

Component	Reaction Mix
KRPG Buffer	44uL
ACO Reagent	2uL
H2O2 Probe	2uL
HRP Reagent	2uL

6. Add 50 uL of Reaction mix to each well.
7. Cover the plate with the aluminum plate seal and incubate the plate at 37oC for 30 minutes.
8. Remove the plate seal and measure the absorbance of the plate at 560 nm using a plate reader.