ab282927 – Free Fatty Acid Colorimetric Assay Kit

For the analysis of fatty acid metabolism and the screening of fatty acids in a variety of samples
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab282927

Storage and Stability

Entire assay kit should be stored at -20°C, protected from light.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer V/Free Fatty Acid Assay Buffer	25 mL	-20°C
OxiRed Probe/Free Fatty Acid Probe	200 µL	-20°C
ACS Reagent (Lyophilized)	1 vial	-20°C
Acyl CoA Enzyme Mix/Enzyme Mix (Lyophilized)	1 vial	-20°C
Enhancer I/Enhancer	200 µL	-20°C
Palmitic Acid Standard (1nmol/µL)	300 µL	-20°C

Materials Required, Not Supplied

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

- 384-well clear plate with flat bottom
- Multi-well spectrophotometer with 384-well plate reading capability

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer V/Free Fatty Acid Assay Buffer: Ready to use. Warm to room temperature prior to use. Store at -20 °C or 4 °C.

OxiRed Probe/Free Fatty Acid Probe: Ready to use as supplied. Warm to room temperature to thaw the OxiRed Probe/Probe solution prior to use. Store at -20°C, Protect from light and moisture. Use within two months.

ACS Reagent: Dissolve in 220 µl of Assay Buffer V/Free Fatty Acid Assay Buffer, pipet up and down to mix. Aliquot and store at -20 °C. Keep on ice while in use. Use within two months.

Acyl CoA Enzyme Mix/Enzyme Mix: Dissolve in 220 µl of Assay Buffer V/Free Fatty Acid Assay Buffer, pipet up and down to mix. Aliquot and store at -20 °C. Keep on ice while in use. Use within two months.

Enhancer I/Enhancer: Ready to use as supplied. Keep on ice while in use. Store at -20°C. Protect from light and moisture. Use within two months.

Palmitic Acid Standard: When stored at -20°C, the Palmitic Acid Standard may separate from the aqueous phase of the solution. To re-dissolve, keep cap tightly closed and heat the Palmitic Acid Standard vial in a hot water bath (80°C-100°C) for 1 min (the standard should look cloudy). Vortex for 30 sec. The standard should now be clear. Repeat the heat and vortexing steps one more time. The Palmitic acid Standard is now completely dissolved in the solution and is ready to use.

Sample Preparation

1. For testing liquid samples, different volumes (1-10 µl) can be added directly to each well in a 384-well plate.
2. Adjust the volume to 11 µl/well with Assay Buffer V/Free Fatty Acid Assay Buffer.
3. Background controls can be performed by preparing parallel sample wells and replacing 2 µl of diluted ACS Reagent with 2 µl Assay Buffer V/Free Fatty Acid Assay Buffer.
4. For testing cell or tissue samples, 10^6 cells or 10 mg tissue samples can be extracted by homogenization with 200 µl of chloroform-Triton X-100 (1% Triton X-100 in pure chloroform) in a micro homogenizer. Then spin the extract 5-10 minutes at top speed in a microcentrifuge.
5. Collect organic phase (lower phase), air dry at 50°C to remove chloroform.
6. Vacuum dry 30 min to remove trace chloroform.
7. Dissolve the dried lipids (in Triton X-100) in 200 µl of Assay Buffer V/ Free Fatty Acid Assay Buffer by vortexing extensively for 5 mins.
8. The solution may be slightly turbid or opalescent, but this does not affect the assay). Use 1-10 µl of the extracted sample per assay.

Δ Notes:

a) For unknown samples, we suggest using different dilutions to ensure that the readings are within the range of the standard curve.
b) Instrument reader settings may need to be adjusted according to the chosen 384-well plate. The correct dimensions of the 384-well plate should be available in the data sheet provided by the plate manufacturer.

Standard Curve Dilution

1. Dilute the Palmitic Acid Standard to 0.25 nmol/µL by adding 25 µl of Palmitic Acid Standard to 75 µl of Assay Buffer V/Free Fatty Acid Assay Buffer, mix well.
2. Add 0, 2, 4, 6, 8, and 10 µl of the diluted Palmitic Acid Standard into a series of wells on a 384-well plate. Adjust the volume to 11 µl/well with Assay Buffer V/Free Fatty Acid Assay Buffer to generate 0, 0.5, 1, 1.5, 2, 2.5 nmol/well of Palmitic Acid Standard.

ACS Reagent

1. Dilute the ACS reagent 1:4 with the Free Fatty Acid Buffer, as needed.
2. Add 2 µl of the diluted ACS Reagent to each standard and sample well.
3. For the background controls, omit the diluted ACS reagent and add 2 µl of the Free Fatty Acid Buffer instead.
4. Mix and incubate 30 min at 37°C to convert Free Fatty Acids into their CoA derivatives.

Reaction Mix:

1. Prepare enough reagent for the number of assays to be performed: For each well, prepare a total 12 µl of Reaction Mix:

Item	Reaction Mix
Assay Buffer V/Free Fatty Acid Assay Buffer	10.5 µL
OxiRed Probe/Free Fatty Acid Probe	0.5 µL
Acyl CoA Enzyme Mix/Enzyme Mix (Lyophilized)	0.5 µL
Enhancer I/Enhancer	0.5 µL

2. Add 12 µl of the Reaction Mix to each well containing the Standards, samples and background control(s). Mix well. Incubate at 37°C for 30 min, protected from light.

Measurement:

Measure absorbance (OD: 570 nm) in a microplate reader.

Calculation

1. Subtract the sample background readings, if significant, from the test sample readings to get the corrected absorbance readings. Otherwise, subtract the 0 Standard reading from the sample readings and the Standard readings.
2. Plot the Palmitic Acid Standard Curve (OD: 570 nm vs nmol Standard).
3. Apply the corrected absorbance readings of the samples to the Palmitic Acid Standard Curve to get the nmol of free Fatty acid in each sample well.

Sample Free Fatty Acid concentration (C) = (FA/V) x D (nmol/µl or mM)

Where: FA = Amount of Free Fatty Acid from Standard Curve (nmol)
V = Sample volume added into the reaction well (µl)
D = Sample dilution factor (if applicable)
Palmitic Acid: 256.43 g/mol
10 mM ≡ 2.56 mg/ml

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)