ab65341 – Free Fatty Acid Assay Kit (Colorimetric/Fluorometric)

For rapid, sensitive and accurate measurement of free fatty acids in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab65341 (use http://www.abcam.cn/ab65341 for China, or http://www.abcam.co.jp/ab65341 for Japan)

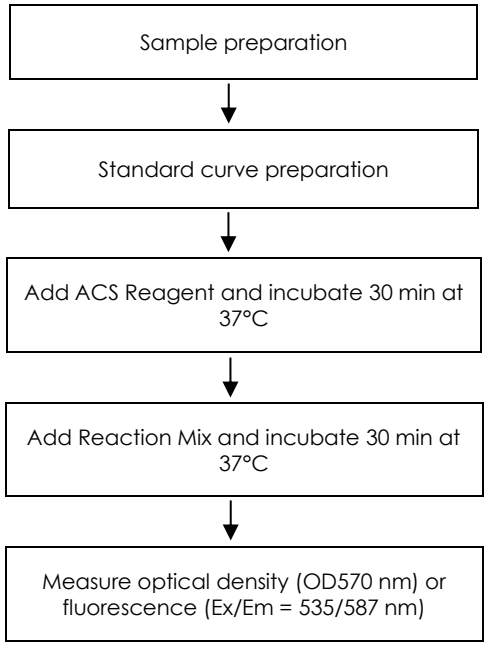
Background:

Free Fatty Acid Assay Kit (ab65341) uses a convenient, sensitive enzyme-based method for detection of medium-to-long-chain free fatty acids in various samples, such as serum, plasma, urine, tissue extracts, cell lysate and cell culture supernatant.

Free fatty acids are converted to their CoA derivatives, which are subsequently oxidized, with colorimetric (570 nm) or fluorometric (Ex/Em 535/587 nm) readout. The kit has a detection limit of 2 µM long-chain fatty acids.

Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.



QUICK ASSAY PROCEDURE

- Preheat incubator and/or plate reader to 37 °C
- Solubilize ACS Reagent and Acyl CoA Enzyme Mix, thaw OxiRed Probe, and re-dissolve the Palmitic Acid Standard; get equipment ready.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Prepare appropriate standard curve for your detection method of choice (colorimetric or fluorometric).
- Set up plate for standard (50 µL) and samples (50 µL).
- Add 2 ml of ACS Reagent into all standard and sample wells.
- Incubate reaction at 37°C for 30 min.
- Prepare Reaction Mix and add 50 µL to each well.
- Incubate plate at 37°C for 30 mins.
- Measure plate at OD 570nm for colorimetric assay or Ex/Em= 535/587 nm

Precautions & Limitations:

NOTE: This kit is not designed for the detection of short-chain fatty acids such as acetic, propionic or butyric acid.

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.

- Modifications to the kit components or procedures may result in loss of performance.
- 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.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Assay Buffer 5	25 mL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
ACS Reagent	1 vial	-20°C	-20°C
Acyl CoA Enzyme Mix	1 vial	-20°C	-20°C
Enhancer I	200 µL	-20°C	-20°C
Palmitic Acid Standard	300 µL	-20°C	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labeled as Assay Buffer V and Fatty Acid Assay Buffer, and OxiRed™ Probe as OxiRed Probe and Fatty Acid Probe (in DMSO, anhydrous). The composition has not changed.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring absorbance (OD) at 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- Microcentrifuge
- 96 well clear plate with clear flat bottom (colorimetric assay) / 96 well black plate with flat bottom (fluorometric assay)
- Triton X-100
- Chloroform
- 1x PBS, pH 7.4
- Vacuum Dryer
- Dounce homogenizer (if using tissues or cells)
- MilliQ water or other type of double distilled/deionized water (ddH_2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Assay Buffer 5 and **Enhancer I:** are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C and protect from light.

OxiRed™ Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and **protect from light and moisture**. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

ACS Reagent and **Acyl CoA Enzyme Mix:** Reconstitute each with 220 µL Assay Buffer 5. Keep on ice during the assay. Use within two months.

Palmitic Acid Standard: Frozen storage may cause Palmitic Acid Standard to separate from the aqueous phase. To re-dissolve, ensure cap is tightly closed (use parafilm or Eppendorf clip, if available) and place in a hot water bath (~80-100°C) for 1 minute or until the standard looks cloudy. Vortex for 30 seconds; the standard should become clear. Repeat the heat and vortex one more time.

Sample Preparation:

1. We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
2. We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.

Cells (adherent or suspension) and Tissue samples:

1. Harvest the number of cells necessary for each assay (initial recommendation = 1 x 10^6 cells or 10 mg tissue).
2. Wash cells in cold PBS.
3. Homogenize in 200 µL chloroform/Triton X-100 (1% Triton X-100 in pure chloroform), using a Dounce homogenizer sitting on ice.
4. Centrifuge sample for 5-10 minutes at 4°C at top speed using a cold microcentrifuge to separate the phases.
5. Collect organic (lower) phase and transfer to a clean tube. Air dry at 50°C in a fume hood to remove the chloroform.
6. Vacuum dry for 30 minutes to remove any remaining trace chloroform.
7. Dissolve the dried lipids in 200 µL of Assay Buffer 5 by vortexing for 5 minutes. The solution may be slightly turbid or opalescent, but this will not affect the assay.
8. Keep on ice.

Liquid Samples (Plasma, Serum, Urine and other biological fluids): Samples can be tested directly or diluted in assay buffer. To find the optimal values we recommend performing several dilutions of the sample (1/2-1/200).

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

For colorimetric assay: Using 1 nmol/ µL (undiluted) Palmitic Acid standard, add 0, 2, 4, 6, 8, 10 µl Palmitic Acid Standard into a series of wells, generating 0, 2, 4, 6, 8 10 nmol/well of Fatty Acid Standard. Adjust the volume to 50 µl/well with Assay Buffer 5. Or use the table below to prepare duplicate standard curve dilutions:

For fluorometric assay: Prepare 0.1 nmol/ µL Palmitic Acid Standard by adding 20 µL of undiluted Palmitic Acid Standard to 180 µL Assay Buffer 5. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.
Using 0.1 nmol/ µL Palmitic Acid standard, add 0, 2, 4, 6, 8, 10 µl Palmitic Acid Standard into a series of wells, generating 0, 0.2, 0.4, 0.6, 0.8 1.0 nmol/well of Fatty Acid Standard. Adjust the volume to 50 µl/well with Assay Buffer 5. Or use the table below to prepare triplicate standard curve dilutions:

Standard #	Volume of 1 nmol/ µL or 0.1 nmol/ µL Standard (µL)*	Assay Buffer 5 (µL)	Final volume standard in well (µL)	End Palmitic Acid Amount (nmoles/well) Colorimetric Assay	End Palmitic Acid Amount (nmoles/well) Fluorometric Assay
1	0	125	50	0	0
2	5	120	50	2	0.2
3	10	115	50	4	0.4
4	15	110	50	6	0.6
5	20	105	50	8	0.8
6	25	100	50	10	1

***NOTE: For colorimetric assay use 1 nmol/ µL standard, for fluorometric assay use 0.1 nmol/ µL standard**

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

1. Set up Reaction wells:
   - Standard wells = 50 µL standard dilutions.
   - Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 5.
2. Add 2 µL of ACS Reagent into all standard and sample wells.
3. Mix well and incubate for 30 minutes at 37°C.
4. Each well (standards, samples, and controls) requires 50 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:
   X µL component x (Number reactions +1).

Component	Colorimetric Assay Reaction Mix (µL)	Fluorometric Assay Reaction Mix (µL)
Assay Buffer 5	44	45.6
OxiRed™ Probe *	2	0.4
Acyl CoA Enzyme Mix	2	2
Enhancer I	2	2

*NOTE: For fluorometric assays, using 0.4 µL/well of the OxiRed™ Probe will reduce background and improve assay sensitivity.

5. Mix Master Reaction Mix by inversion. Add 50 µL of the Master Reaction Mix to each well. Use a clean tip for each well.
6. Mix and incubate at 37°C for 30 minutes, protected from light.
7. Measure output immediately on a microplate reader at OD 570 nm for Colorimetric assay or Ex/Em= 535/587 nm for Fluorometric assay.

Calculations:

1. Average the replicate readings for each standard and sample and background control (if required).
2. Subtract the mean value of the blank (Standard #1) from all standard and sample readings. This is the corrected value for absorbance or fluorescence.
3. Plot the corrected values for each standard as a function of the final amount of Palmitic Acid. Calculate the equation based on the corrected standard curve data using a linear regression and determine the slope.
4. Interpolate the amount of Fatty Acid (B) in the sample wells by using the linear standard curve equation.
5. Concentration of Free Fatty Acid in the test samples is calculated as:

$$Fatty\ Acid\ Concentration = \left(\frac{B}{V}\right) \times D = nmol/\mu L = mM$$

Where:
B = amount of Fatty Acid in the sample well calculated from standard curve (in nmoles).
V = sample volume added in sample wells (in µL).
D = sample dilution factor (before addition to the well).

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
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