Ab65341 – Free Fatty Acid Assay Kit - Quantification

For rapid, sensitive and accurate measurement of Free Fatty Acid in various 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/ab65341 (use http://www.abcam.cn/ab65341 for China, or http://www.abcam.co.jp/ab65341 for Japan)

Storage and Stability: Store kit at -20°C in the dark immediately upon receipt.

Materials Supplied:

Item	Quantity	Storage temperature
Assay Buffer V/Fatty Acid Assay Buffer	25 mL	-20°C
OxiRed Probe/Fatty Acid Probe (in DMSO)	200 µL	-20°C
ACS Reagent/Acyl-CoA Synthetase (ACS) Reagent	1 vial	-20°C
Acyl CoA Enzyme Mix/Enzyme Mix	1 vial	-20°C
Enhancer I/Enhancer	200 µL	-20°C
Palmitic Acid Standard (1 nmol/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:

- Microplate reader capable of measuring absorbance at OD 570 nm (colorimetric) or fluorescence at Ex/Em = 535/587 nm (fluorometric)
- MilliQ water or other type of double distilled water (ddH2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom (colorimetric assay) / 96 well plate with clear flat bottom, preferably black (fluorometric assay)
- Dounce homogenizer (if using tissue)
- Triton X-100
- Chloroform: chloroform is potentially hazardous, so please use in a well-ventilated area or fume hood.
- Vacuum dryer

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 V/Fatty Acid Assay Buffer and **Enhancer I/Enhancer** are ready to use as supplied.

OxiRed Probe/Fatty Acid Probe: Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep on ice during the assay. Store aliquots protected from light and moisture. Use within two months.

ACS Reagent/Acyl-CoA Synthetase (ACS) Reagent and **Acyl CoA Enzyme Mix/Enzyme Mix:** Reconstitute each with 220 µL Assay Buffer V/Assay Buffer. Keep on ice during the assay. Use within two months.

Palmitic Acid Standard (1 nmol/µL): Keeping the cap tightly closed, 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.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Discard the working standard dilutions after use as they do not store well.
- Each dilution has enough amount of standard to set up duplicate readings (2 x 50 µL).
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

For colorimetric assay: Using the Palmitic Acid Standard (1 nmol/µL), prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes.
For fluorometric assay: Prepare a 0.1 nmol/µL Palmitic Acid standard by diluting 20 µL of the 1 nmol/µL Standard with 180 µL of Assay Buffer V/Assay Buffer. Using 0.1 nmol/µL standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Palmitic Acid Standard (µL)	Assay Buffer V/Assay Buffer (µL)	Final volume standard in well (µL)	End Palmitic acid Conc in well (nmol/well) Colorimetric Assay	End Palmitic acid Conc in well (nmol/well) Fluorometric Assay
1	0	150	50	0	0
2	6	144	50	2	0.2
3	12	138	50	4	0.4
4	18	132	50	6	0.6
5	24	126	50	8	0.8
6	30	120	50	10	1

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

- 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.

Cell (adherent or suspension) and Tissue samples:

1. Harvest the amount of cells or tissue necessary for each assay (initial recommendation = 1 x 10^6 cells or 10 mg tissue).
2. Wash with cold PBS.
3. Homogenize in 200 µL chloroform/Triton X-100 (1% Triton X-100 in pure chloroform).
   - Homogenize cells by pipetting up and down or using a micro-homogenizer.
   - Homogenize tissue using a micro-homogenizer or Dounce homogenizer.
4. Incubate on ice 10 – 30 minutes.
5. Spin the extract for 5 – 10 minutes at top speed in a microcentrifuge.
6. Collect organic phase (lower phase), air dry at 50°C in a fume hood to remove chloroform.
7. Vacuum dry for 30 minutes to remove trace chloroform.
8. Dissolve the dried lipids in 200 µL of Assay Buffer V/Fatty Acid Assay Buffer by vortexing extensively for 5 minutes. NOTE: The solution may be slightly turbid or opalescent, but this does not affect the assay.

Liquid Samples (plasma, serum, urine and other biological fluids): Liquid samples can be tested directly by adding sample to the microplate wells. To find the optimal values we recommend performing several dilutions of the sample in a range (1/2 – 1/200).

Assay Procedure:

- Equilibrate all 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 V/Assay Buffer).
2. Add 2 µL of ACS Reagent into all standard and sample wells.
3. Mix and incubate the reaction for 30 minutes at 37°C.
4. Prepare 50 µL Reaction Mix for each reaction to be performed: standards, samples and controls. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation:
   X µL component x (Number reactions +1).

Component	Colorimetric Assay Reaction Mix (µL)	Fluorometric Assay Reaction Mix (µL)
Assay Buffer V/Assay Buffer	44	45.6
OxiRed Probe/Fatty Acid Probe*	2	0.4
Acyl CoA Enzyme Mix/Enzyme Mix	2	2
Enhancer I/Enhancer	2	2

*NOTE: For fluorometric readings, using 0.4 µL/well of the OxiRed Probe/Fatty Acid probe decreases the background readings, therefore increasing detection sensitivity.

5. Add 50 µL of Reaction Mix to each well.
6. 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:

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of Fatty Acid.
4. Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).
5. Concentration of Free Fatty Acid in the test samples is calculated as:

$$Fatty\ Acid\ Concentration = \left(\frac{Fa}{Sv}\right) * D$$

Where:
Fa = amount of fatty acid in the sample well calculated from standard curve (nmol).
Sv = amount of sample volume added in sample wells (µL).
D = sample dilution factor.

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)

To get Chinese protocol, please scan QR code via WeChat or visit Protocols section at www.abcam.cn/ab65341