Free Fatty Acid Colorimetric Assay Kit (384-well) (#BN01116)

(Catalog #BN01116; 400 assays; Store at -20°C)

I. Introduction:

Fatty Acids play important roles in normal metabolism and in many disease states. They are precursors to a number of bioactive classes of compounds including prostaglandins and leukotrienes, and have been implicated to play roles in such diverse functions as the immune system, autism, and the inflammatory response. Fatty acid levels are an important measure of the physiologic state. High levels are associated with diabetes mellitus, obesity, endocrine dysfunctions, and metabolic syndrome. Assay Genie's Free Fatty Acid Colorimetric assay kit provides a simple method for the quantification free fatty acids. The kit offers a convenient, sensitive, enzyme-based method for the detection of long-chain (C-8 (octanoate) and longer) fatty acids in various biological samples. In the assay, fatty acids are converted to their CoA derivatives, which are subsequently oxidized by the enzyme mix to yield an intermediate. Formed intermediate then reacts with the Free Fatty acid probe to generate color which can be read by a spectrophotometer at OD 570 nm. Our 384-well format enables analyses of large number of samples on a single plate in a high throughput mode. Sample volumes between 1 and 10 µl can be used with this kit.

II. Applications:

- High throughput screening for fatty acids in a variety of samples
- Analysis of fatty acid metabolism

III. Sample Types:

- Serum, plasma, and other body fluids
- Animal tissues
- Cell culture: adherent or suspension cells
- Growth media and foods

IV. Kit Contents:

Components	BN01116	Cap Code	Part Number
Free Fatty Acid Assay Buffer	25 ml	WM	BN01116-1
Free Fatty Acid Probe (in DMSO, anhydrous)	200 µl	Red	BN01116-2A
ACS Reagent (lyophilized)	1 vial	Blue	BN01116-3
Enzyme Mix (lyophilized)	1 vial	Green	BN01116-4
Enhancer	200 µl	Purple	BN01116-5
Palmitic Acid Standard (1 nmol/µl)	300 µl	Yellow	BN01116-6

V. User Supplied Reagents and Equipment:

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

VI. Storage and Handling:

Store kit at –20° C, protect from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay. Keep enzymes on ice while using the kit.

VII. Reagent Preparation and Storage Conditions:

- **Free Fatty Acid Assay Buffer:** Ready to use. Warm to room temperature prior to use. Store at -20 °C or 4 °C.
- **Free Fatty Acid Probe:** Ready to use as supplied. Warm to room temperature to thaw the 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 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.
- **Enzyme Mix:** Dissolve in 220 µl of 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:** 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 it, keep the 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.

VIII. Free Fatty Acid Assay Protocol:

1. **Sample Preparation:** For testing liquid samples, different volumes (1-10 µl) can be added directly to each well in a 384-well plate. Adjust the volume to 11 µl/well with Free Fatty Acid Assay Buffer. Background controls can be performed by preparing parallel sample wells and replacing 2 µl of diluted ACS Reagent with 2 µl Free Fatty Acid Assay Buffer (see step 3 "ACS Reagent"). 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 microhomogenizer. Then spin the extract 5-10 minutes at top speed in a microcentrifuge. Collect organic phase (lower phase), air dry at 50°C to remove chloroform. Vacuum dry 30 min to remove trace chloroform. Dissolve the dried lipids (in Triton X-100) in 200 µl of Fatty Acid Assay Buffer by vortexing extensively for 5 mins.

   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.

Notes:

1. For unknown samples, we suggest using different dilutions to ensure that the readings are within the range of the standard curve.
2. 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.

2. **Standard Curve Preparation:** Dilute the Palmitic Acid Standard to 0.25 nmol/µL by adding 25 µl of Palmitic Acid Standard to 75 µl of Free Fatty Acid Assay Buffer, mix well. 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 Free Fatty Acid Assay Buffer to generate 0, 0.5, 1, 1.5, 2, 2.5 nmol/well of Palmitic Acid Standard.
3. **ACS Reagent:** Dilute the ACS reagent 1:4 with the Free Fatty Acid Buffer, as needed. Add 2 µl of the diluted ACS Reagent to each standard and sample well. For the background controls, omit the diluted ACS reagent and add 2 µl of the Free Fatty Acid Buffer instead. Mix and incubate 30 min at 37°C to convert Free Fatty Acids into their CoA derivatives.
4. **Free Fatty Acid Reaction Mix:** Prepare enough reagent for the number of assays to be performed: For each well, prepare a total 12 µl of Reaction Mix:

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	Reaction Mix
Free Fatty Acid Assay Buffer	10.5 µl
Free Fatty Acid Probe	0.5 µl
Enzyme Mix	0.5 µl
Enhancer	0.5 µl

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.

5. **Measurement:** Measure absorbance (OD: 570 nm) in a microplate reader.
6. **Calculations:** 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. Plot the Palmitic Acid Standard Curve (OD: 570 nm vs nmol Standard). 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** is the amount of Free Fatty Acid from Standard Curve (nmol)
V is the sample volume added into the reaction well (µl)
D is the sample dilution factor (if applicable)

Palmitic Acid: 256.43 g/mol
10 mM ≡ 2.56 mg/ml

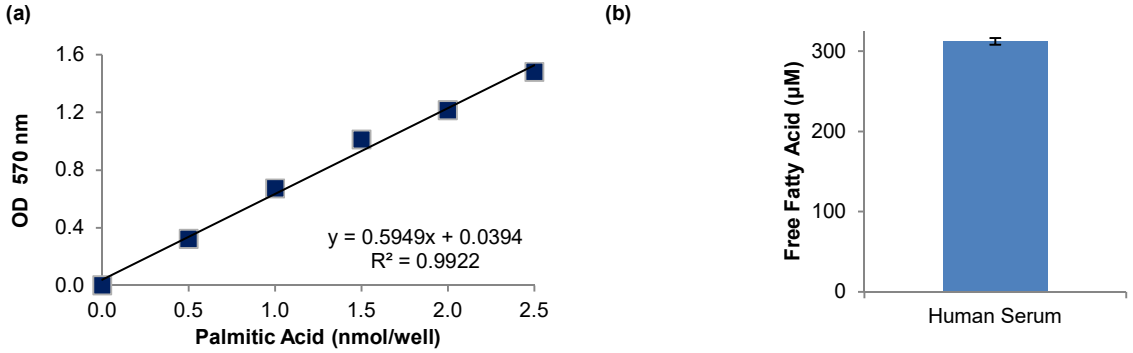

Figure: (a) Palmitic Acid Standard Curve. (b) Determination of Free Fatty Acid in pooled normal human serum. Undiluted serum sample (2 µl, untreated) was assayed directly according to the kit protocol. Calculated Free Fatty Acid concentration: 312 ± 4.2 µM.

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