Human Free fatty acids (FFA) ELISA Kit

Cat #: orb1670252 (manual)

For research use only. Not for use in diagnostic or therapeutic procedures.

REAGENTS AND MATERIALS PROVIDED

Reagents	Quantity	Reagents	Quantity
Assay plate (96 Wells)	1	Instruction manual	1
Standard (lyophilized)	2	Sample Diluent	1 x 20 mL
Biotin-Conjugate (concentrate 100 x)	1 x 120 µL	Biotin-Conjugate Diluent	1 x 20 mL
Streptavidin-HRP (concentrate 100 x)	1 x 120 µL	Streptavidin-HRP Diluent	1 x 20 mL
Wash Buffer (concentrate 25 x)	1 x 20 mL	Substrate Solution	1 x 12 mL
Stop Solution	1 x 10 mL	Adhesive Films	4

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
2. Precision single or multi-channel pipettes and disposable tips.
3. Deionized or distilled water.
4. Eppendorf Tubes for serial dilution samples.
5. Container for Wash Solution.
6. Absorbent paper for blotting the microtiter plate.

STORAGE

<table>
<tr><td>Unopened kit</td><td colspan="2">Store at 2 - 8°C. If not for recent use, better keep it store at -20°C. Do not use past kit in expiration date.</td></tr>
<tr><td rowspan="10">Opened kit</td><td>Coated assay plate</td><td>May be stored for up to 1 month at 2 - 8°C. Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal, and avoid the damp.</td></tr>
<tr><td>Standard</td><td rowspan="9">May be stored for up to 1 month at 2 - 8°C. If not for recent use, better keep it store at -20°C.</td></tr>
<tr><td>Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP</td></tr>
<tr><td>Sample Diluent</td></tr>
<tr><td>Biotin-Conjugate Diluent</td></tr>
<tr><td>Streptavidin-HRP Diluent</td></tr>
<tr><td>Wash Buffer</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

***Once the standard is reconstituted it must be used immediately and cannot be stored for repeated use.**

INTRODUCTION

In chemistry, particularly in biochemistry, a fatty acid is a carboxylic acid with a long aliphatic chain, which is either saturated or unsaturated. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28. Fatty acids are usually derived from triglycerides or phospholipids. Fatty acids are important sources of fuel because, when metabolized, they yield large quantities of ATP. Many cell types can use either glucose or fatty acids for this purpose. Long-chain fatty acids cannot cross the blood brain barrier (BBB) and so cannot be used as fuel by the cells of the central nervous system; [citation needed] however, free short-chain fatty acids and medium-chain fatty acids can cross the BBB, in addition to glucose and ketone bodies.

PRINCIPLE OF THE ASSAY

This assay employs a two-site sandwich ELISA to quantitate FFA in Human serum, plasma. An antibody specific for FFA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any FFA present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for FFA is added to the wells. After washing, Streptavidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and

color develops in proportion to the amount of FFA bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the
- samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the ELISA Kit, the possibility of interference cannot be excluded.

DETECTION RANGE

31.25 µg/mL - 2000 µg/mL. The standard curve concentrations used for the ELISA's were 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 0 µg/mL.

SENSITIVITY

The limit of detection of Human FFA defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 15.6 µg/mL (mean of 6 independent assays).

SPECIFICITY

This assay has high sensitivity and excellent specificity for detection of Human FFA. No significant cross-reactivity or interference between Human FFA and analogues was observed.

Note:

Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between Human FFA and all the analogues, therefore, cross reaction may still exist.

PRECISION

Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples of known concentration were tested in forty separate assays to assess inter-assay precision.

CV (%) = SD/meanX100

Intra-Assay: CV<8%

Inter-Assay: CV<12%

STABILITY

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 5% within the expiration date under appropriate storage condition.

The loss rate was determined by accelerated thermal degradation test. Keep the kit at 37°C for 4 and 7 days, and compare O.D.values of the kit kept at 37 °C with that of at recommended temperature. (Referring from China Biological Products Standard, which was calculated by the Arrhenius equation. For ELISA kit, 4 days storage at 37 °C can be considered as 6 months at 2-8 °C, which means 7 days at 37°C equaling 12 months at 2-8°C).

Note:

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

SAMPLE COLLECTION AND STORAGE

- **Serum** Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 2-8 °C before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
- **Plasma** Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2-8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
- **Other biological fluids** Centrifuge samples for 20 minutes at 1000 × g. Remove particulates and assay immediately or store samples in aliquot at -20°C or -80 °C. Avoid repeated freeze/thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
2. Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.
3. When performing the assay, bring samples to room temperature.

SAMPLE PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer (1 x) - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25 x) into deionized or distilled water to prepare 500 mL of Wash Buffer (1 x).

Biotin-Conjugate (1 x) - Centrifuge the vial before opening.

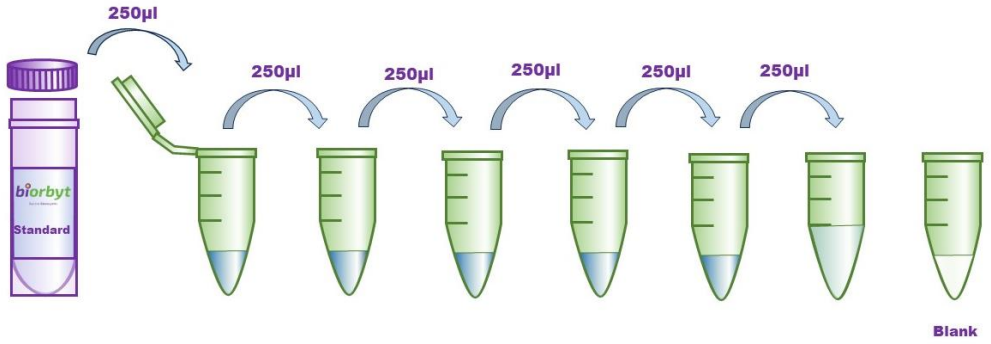
Biotin-Conjugate requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Biotin-Conjugate (100 x) + 990 µL of Biotin-Conjugate Diluent.

Streptavidin-HRP (1 x) - Centrifuge the vial before opening.

Streptavidin-HRP requires a 100-fold dilution. A suggested 100-fold dilution is 10 µL of Streptavidin-HRP (100 x) + 990 µL of Streptavidin-HRP Diluent.

FFA Standard - Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1 mL of Sample Diluent. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 2000 µg/mL). The standard has to be used immediately after reconstitution and cannot be stored.

Use Eppendorf Tubes - Pipette 250 µL of the Sample Diluent into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted standard serves as the high standard (2000 µg/mL). The Sample Diluent serves as the zero standard (0 µg/mL).



Tube	S7	S6	S5	S4	S3	S2	S1	S0
µg/mL	2000	1000	500	250	125	62.5	31.25	0

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.

2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2 - 8°C.

3. Add 100 µL of standard and sample per well. Cover with the adhesive films provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.

4. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µL of Biotin-Conjugate (1 x) to each well. Cover with the adhesive films. Incubate for 1 hour at 37°C. (Biotin-Conjugate (1 x) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)

6. Aspirate each well and wash, repeating the process for a total of three washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

7. Add 100 µL of Streptavidin-HRP (1 x) to each well. Cover the microtiter plate with the adhesive films. Incubate for 1 hour at 37°C.

8. Aspirate each well and wash, repeating the process for a total of five washes. Wash by filling each well with Wash Buffer (250 µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

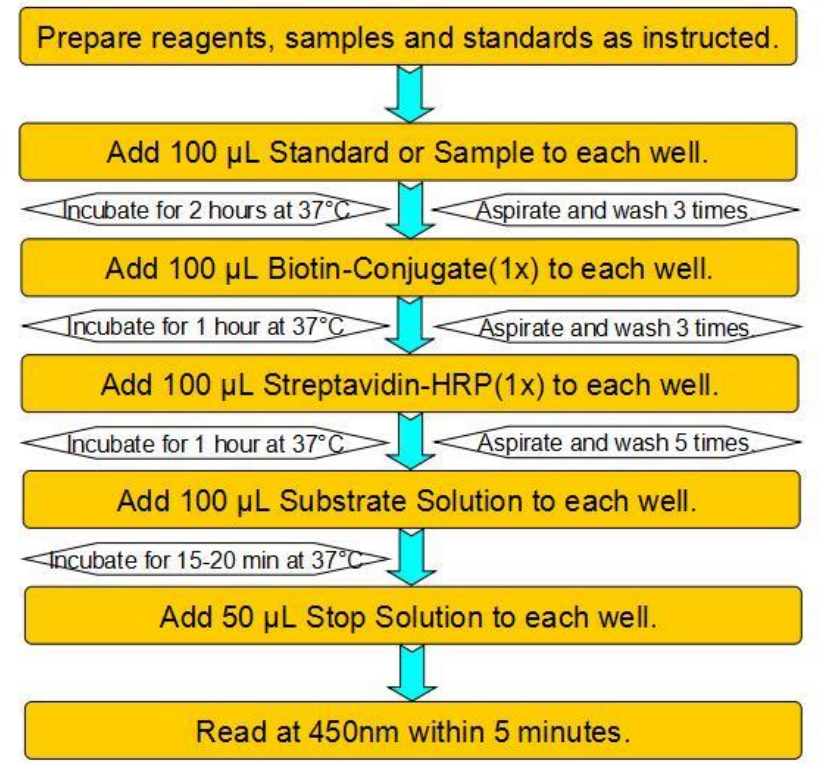
9. Add 100 µL of Substrate Solution to each well. Incubate for 15-20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark. Avoid placing the plate in direct light.

10. Add 50 µL of Stop Solution to each well. When the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

11. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. Subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

***Samples may require dilution. See Sample Preparation section.**

ASSAY PROCEDURE SUMMARY



***Samples may require dilution. Please refer to sample preparation section.**

CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the FFA concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

IMPORTANT NOTE

1. The instruction manual also suits for the kit of 48T, but all reagents of 48T kit are reduced by half.

2. There may be some foggy substance in the wells when the plate is opened at the first time. It will not effect on the final assay results. Do not remove microtiter plate from the storage bag unless needed.

3. Do not mix or substitute reagents from one kit lot to another. Use only the reagents supplied by manufacturer.

4. Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as far as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

5. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.

6. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.

7. Controlling of reaction time: Observe the change of color after adding Substrate Solution (e.g. observation once every 10 minutes), Substrate Solution should change from colorless or light-blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

8. Substrate Solution is easily contaminated. Substrate Solution should remain colorless or light blue until added to the plate. Please protect it from light.

9. Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

10. Protect all reagents from strong light during storage and incubation. All the bottle caps of reagents should be covered tightly to prevent the evaporation and contamination of microorganism.

11. Wrong operations during the reagents preparation and loading, as well as incorrect parameter setting for the plate reader may lead to incorrect results. A microplate plate reader with a bandwidth of 10nm or less and an optical density range of 0-3 O.D. or greater at 450 ± 10nm wavelength is acceptable for use in absorbance measurement.

12. Even the same operator might get different results in two separate experiments. In order to get better reproducible results, the operation of every step in the assay should be controlled. Furthermore, a preliminary experiment before assay for each batch is recommended.

13. Limited by the current condition and scientific technology, we can't completely conduct the comprehensive identification and analysis on the raw material provided by suppliers. So, there might be some qualitative and technical risks to use the kit.

14. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments. Please make sure that sufficient samples are available.

15. Kits from different batches may be a little different in detection range, sensitivity and color developing time.

16. Each kit has been strictly passed Q.C test. However, results from end users might be inconsistent with our in-house data due to some unexpected transportation conditions or different lab equipments. Intra-assay variance among kits from different batches might arise from above factors, too.

17. Kits from different manufacturers with the same item might produce different results, since we haven't compared our products with other manufacturers.

18. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

19. Valid period: six months.