HUMAN ADIPOCYTE FATTY ACID BINDING PROTEIN (AFABP/FABP-4) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN AFABP CONCENTRATIONS IN SERUM AND PLASMA

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	HUMAN AFABP/FABP-4 ELISA
Catalog No.	SK00030-09
Lot No.	
Formulation	96 T
Standard range	1.56 - 50 ng/mL
Sensitivity	0.1-0.2 ng/mL
Sample Volume	100 µL
Sample Type	Serum, Plasma
Dilution factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human AFABP/FABP4
Calibration	Human AFABP/FABP4 Recombinant
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8°C for 1 month, more information check page 2 and 3
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

THIS PROTOCOL AND DATA IS PROVIDED FOR DEMONSTRATION ONLY.
ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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DESCRIPTION

This Human AFABP ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human AFABP from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human AFABP and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural AFABP samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human AFABP. The capture antibody can bind to the human AFABP in the standard and samples. After washing the plate of any unbound substances, an antibody against human AFABP is added to the wells. After another washing of the plate, Anti Rabbit IgG-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human AFABP bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
AFABP Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with an antibody against AFABP.	**030-09-01**	**1 plate**
AFABP Standard – refer to lot of recombinant human AFABP in a buffered protein base with preservative; lyophilized.	**030-09-02**	**1 vial**
Detection Antibody Concentrate – refer to lot of vial, 50-fold concentrate of polyclonal antibody against AFABP in liquid.	**030-09-03**	**1 vial**
Positive Control - one vial of recombinant human AFABP in a buffered protein base with preservative; lyophilized.	**030-09-04**	**1 vial**
ARIGHRP Conjugate - 120 µl/vial, 100-fold concentrated solution of Goat Anti Rabbit IgG HRP conjugate.	**ARIGHRP**	**1 vial**
Dilution Buffer - 40 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Antibody & HRP Diluent Solution - 30 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution- 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 1 month. After receipt of ELISA kit, open box and remove Detection Antibody and store at -20°C until ready to use. For longer storage up to 8 months, unopened Standard, Positive Control, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at

-20° C. ARIGHRP Conjugate and TMB Substrate Solution should be stored at 2 ~ 8° C. Do not use kit past expiration date.

Do not use kit past expiration date. ARIG-HRP Conjugate 100-fold concentrated solution (protect from light) may be stored at 2 – 8° C for up to 8 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8° C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Samples DO NOT need to be diluted. **Optimal dilutions should be determined by each laboratory for each application. Use polypropylene test tubes.**

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

AFABP Standard - Reconstitute the AFABP standard with refer to lot of Dilution Buffer. Pipette 250 µL of Dilution Buffer into tubes #2 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **50 ng/mL** standard serves as the high standard. The appropriate Dilution Buffer serves as the zero standard (0 ng/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Refer to lot	
# 1	Refer to lot	Refer to lot	50 ng/ml
# 2	250 µl of 1	250 µl	25 ng/ml
# 3	250 µl of 2	250 µl	12.5 ng/ml
# 4	250 µl of 3	250 µl	6.25 ng/ml
# 5	250 µl of 4	250 µl	3.125 ng/ml
# 6	250 µl of 5	250 µl	1.56 ng/ml

Positive Control - Reconstitute the positive control with refer to lot of Dilution Buffer to make positive control solution.

Detection Antibody - Pipette refer to lot of **Antibody & HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer refer to lot of 50-fold concentrated stock solution to prepare working solution.

ARIGHRP Conjugate - Pipette 11.88 mL of **Antibody & HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold

concentrated stock solution to prepare working solution. *(protect from light).*

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells.
4. Add 100 µL of **Standard solutions** #6 to # 1, samples, or positive control per well. Cover with plate sealer. Incubate for 2 hours on a microplate shaker at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 µL) using a squirt bottle, 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.
6. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of **ARIGHRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a microplate reader set to 450 nm.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log curve fit to more accurately quantify the standard dilutions.

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

TYPICAL STANDARD CURVE

This standard curve data is provided for demonstration only. A new standard curve should be generated for each set of samples assayed.

AFABP (NG/ML)	CORRECTED (450NM)
Blank	0 (refer to lot)
1.563	0.064
3.125	0.122
6.25	0.263
12.5	0.522
25	1.055
50	2.224

SPECIFICITY

PROTEINS	CROSSREACTIVITY (%)
Human AFABP	100
Human FABP3	0
Human FABP7	0
Human sCD36	0
Human FTO	0
Human ADRP	0

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Anti Rabbit IgG HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light**.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate refer to lot on the plate shaker at RT. **Protect from light**.
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min.