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

FOR THE QUANTITATIVE DETERMINATION OF RAT OR MOUSE AFABP CONCENTRATIONS IN SERUM AND PLASMA

PURCHASE INFORMATION:

ELISA NAME	RAT/MOUSE AFABP/FABP-4 ELISA
Catalog No.	SK00030-03
Lot No.:	
Formulation	96 T
Standard range	3.2-2000 ng/mL
Sensitivity	0.64 ng/mL
Sample Volume	50 µl
Dilution Factor	*2~4 (Optimal dilutions should be determined by each laboratory for each application)*
Sample Type	Serum, EDTA plasma
Specificity	Rat, Mouse, Human
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 12%
Storage	4 °C

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INTRODUCTION

Rat AFABP ELISA employs the quantitatively competitive enzyme immunoassay technique in which rat AFABP present in samples competed with a fixed amount of biotinylated rat AFABP for sites on purified rabbit IgG specific against rat AFABP. During the incubation, the rabbit IgG becomes bound to the goat anti-rabbit IgG pre-coated onto the microplates. Following a wash to remove any unbound antibody, standard, samples and biotin conjugate, a Streptavidin conjugated to horseradish-peroxidase (HRP) is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when Stop Solution is added. The intensity of the color measured is in inverse proportion to the amount of rat AFABP bound in the initial step. The sample values are then read off the standard curve.

Rat AFABP ELSA has been shown to accurately quantify the recombinant and natural rat AFABP. Results obtained using natural rat AFABP showed dose response curves that were parallel to the standard curves obtained using the kit standards.

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 Dilution Buffer 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 Dilution Buffer 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 immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
R-Microplate – 96 well microplate precoated with polyclonal anti-rabbit IgG	**RM01**	**1 plate**
AFABP Standard – 10 µg/vial of recombinant rat AFABP in a buffered protein base with preservatives; lyophilized.	**030-03-01**	**1 vial**
AFABP Biotin Concentrate - 175 µL/vial, 10-fold Concentrate of AFABP biotinylated with preservatives; lyophilized.	**030-03-02**	**2 vials**
AFABP Antibody Concentrate – 175 µL/vial, 10-fold concentrated of polyclonal purified IgG against rat AFABP with preservatives; lyophilized.	**030-03-03**	**2 vials**
Positive Control – one vial of recombinant rat AFABP, lyophilized (optional)	**030-03-04**	**1 vial**
Streptavidin-HRP Conjugate - 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	**DB18**	**1 bottle**
HRP Diluent Solution - 12 mL of buffered protein based solution with preservates	**DB06**	**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**
Plastic Pouch	**P01**	**1**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 6 months. For longer storage, unopened Standard, Positive Control, Antibody Concentrate and Biotin Concentrate should be stored at -20 or -70°C. Do not use kit past expiration date.

Opened / Reconstituted Reagents: Reconstituted Standard, Biotin Solution and Antibody Solution SHOULD BE STORED at -20 °C or -70°C for up to one month. Reconstituted Biotin Solution CAN NOT BE STORED at 2-8°C. Streptavidin-HRP Conjugate 100-fold concentrated and other components may be stored at 2 - 8°C for up to 6 months.
Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

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 1000 x 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 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

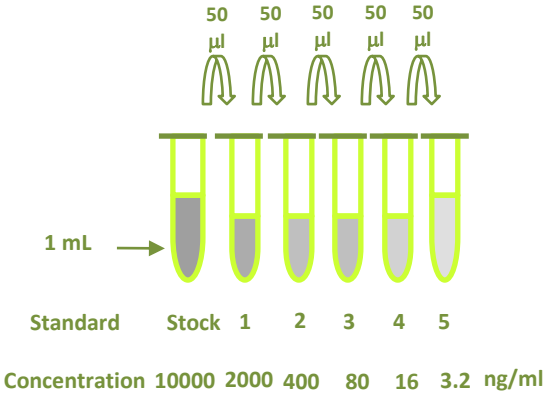
SAMPLE PREPARATION

Serum and plasma samples require a 2-4 fold dilution. A suggested 2-fold dilution is 60 µL sample + 60 µL Dilution Buffer. A suggested 4-fold dilution is 30 µL sample + 90 µL Dilution Buffer. **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 Wash Buffer.
AFABP Standard - Refer to vial label for reconstitution volume. Reconstitute the **AFABP** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 10,000 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 200 µL of Dilution Buffer into tubes #1 to #5. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 ng/mL** standard serves as the high standard.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 ml	10000 ng/ml
# 1	50µl of stock	200µl	2000 ng/ml
# 2	50µl of 1	200µl	400 ng/ml
# 3	50µl of 2	200µl	80 ng/ml
# 4	50µl of 3	200µl	16 ng/ml
# 5	50µl of 4	200µl	3.2 ng/ml



AFABP Antibody Concentrate - Reconstitute the Antibody Concentrate with 175 µL of Dilution Buffer to produce a 10-fold concentrated stock solution. Transfer it to 1.575 mL of Dilution Buffer to prepare 1x Antibody Solution. **Note:** This is enough for half a plate, 2 vials of AFABP Antibody Concentrate are included with this kit.

AFABP Biotin Concentrate - Reconstitute the Biotin Concentrate with 175 µL of Dilution Buffer to make 10-fold concentrated solution. Transfer it to 1.575 mL of Dilution Buffer to prepare 1x Biotin Solution. **Note:** This is enough for half a plate, 2 vials of AFABP Biotin Concentrate are included with this kit.

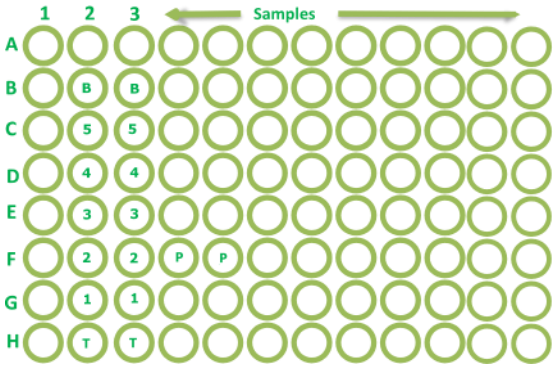
Streptavidin-HRP Conjugate - Transfer 120 µl of 100-fold concentrated stock solution to 11.88 mL of **HRP Diluent Solution** (DB06) to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave well B2 and B3 as Blank. **DO NOT ADD ANY ANTIBODY OR BIOTINYLATED SOLUTION INTO BLANK WELLS.**
4. Set H2 and H3 as total binding. Add 50 µl per well of Dilution Buffer.
5. Add 50 µl per well of standard solution from #5 to #1 (reverse order of serial dilution) to the appropriate wells (C2, C3 to G2, G3). Add 50 µl per well of Positive control (PC) into wells F4 and F5. Add 50 µl per well of samples into appropriate wells.
6. Add 25µl per well of 1x Antibody solution into total binding, standard, PC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). Note: Standard, Blank and PC should be assayed in duplicates. **DO NOT ASPIRATE AND WASH. PROCEED IMMEDIATELY TO THE NEXT STEP.**
7. Add 25 µl per well of 1x Biotin Solution into total binding, standard, PC and samples wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker.
8. Aspirate wells and wash 4 times with 300 µl of 1x Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
9. Add 100 µL of Streptavidin-HRP Conjugate working solution. Cover or seal the plate and incubate at room temperature for 60 minutes on microplate shaker. **Protect from light**.
10. Repeat the aspiration/wash as in step 8.
12. Add 100 µL of Substrate Solution to each well. Incubate for 3-6 minutes at room temperature on microplate shaker. **Protect from light**.
13. 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 micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, PC, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a four parameter logistic (4-PL) curve-fit. The standard curve shows relationship between standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

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

WELL	OD450 READING	STANDARD (NG/ML)
Blank	0.102	
Total Binding	1.074	0
Standard 5	1.062	3.2
Standard 4	0.778	16
Standard 3	0.377	80
Standard 2	0.174	400
Standard 1	0.108	2000

- Lot No.:
- Positive Control: 20 - 40 ng/mL

CALIBRATION

This immunoassay is calibrated against a highly purified *E. Coli*-expressed recombinant rat AFABP.

SENSITIVITY

The minimum detectable dose (MDD) of rat AFABP was 0.64 ng/mL.

SPECIFICITY

This assay recognizes both natural and recombinant rat AFABP/FABP-4. The data also indicated that mouse serum samples were competitively bound to antibody that was used in this kit formulation condition. Its linear dilution curves were parallel to the standard curves obtained using the ELISA standard. That means mouse serum samples cross-react with rat AFABP/FABP-4 ELISA kit.

PROTEINS	CROSS-REACTIVITY
Rat AFABP	100%
Mouse AFABP	100%
Human AFABP	100%
Human FABP-3	0
Human FABP-7	0

LINEARITY

To assess the linearity of the assay, pooled research mouse serum samples were diluted with Dilution Buffer DB18 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
2X	47.199	94.398	100
10X	9.220	92.20	97.7

To assess the linearity of the assay, pooled research mouse EDTA plasma samples were diluted with Dilution Buffer DB18 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
2X	28.289	56.578	100
10X	4.954	49.54	87.6

To assess the linearity of the assay, pooled research rat serum samples were diluted with Dilution Buffer DB18 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
2X	21.921	43.842	100
10X	4.819	48.19	110

To assess the linearity of the assay, pooled research rat EDTA plasma samples were diluted with Dilution Buffer DB18 and assayed.

DILUTION FACTOR	ASSAYED (NG/ML)	FINAL (NG/ML)	RECOVERY (%)
2X	18.851	37.702	100
10X	3.725	37.25	98.8

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 50 µl of standard, samples, positive control to wells. Add 25 µL of 1x Antibody Solution to each well, except for blanks. Incubate 2 hours on the plate shaker at RT. **DO NOT ASPIRATE AND WASH BEFORE ADDING 1x BIOTIN SOLUTION.**
↓
Add 25 µl 1x Biotin Solution to each well, except for blanks. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 3-6 min on the bench top. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min