

Mouse Adipocyte FABP ELISA (Mouse FABP4 ELISA, Mouse aP2 ELISA)

Cat. No. RD291036200R

Manufacturer

BioVendor - Laboratorní medicína a.s.

Place of business:

CTPark Modrice
Evropska 873
664 42 Modrice
CZECH REPUBLIC
e-mail: info@biovendor.com
<http://www.biovendor.com>
Phone: +420-549 124 185
Fax: +420-549 211 460

In USA distributed by:

**Immuno-Biological Laboratories, Inc.
(IBL-America)**
8201 Central Ave NE, Suite P
Minneapolis, MN 55432 USA

Phone: 763-780-2955 888-523-1246
Fax: 763-780-2988
e-mail: mkowal@ibl-america.com
www.ibl-america.com

CONTENTS:

1. INTENDED USE.....	3
2. STORAGE, EXPIRATION	3
3. SUMMARY	4
4. TEST PRINCIPLE	5
5. PRECAUTIONS	5
6. REAGENTS SUPPLIED	6
7. MATERIALS REQUIRED BUT NOT SUPPLIED	6
8. PREPARATION OF REAGENTS.....	7
9. PREPARATION OF SAMPLES	8
10. ASSAY PROCEDURE	9
11. CALCULATIONS.....	10
12. LIMITS OF ASSAY	11
13. PERFORMANCE CHARACTERISTICS	11
14. DEFINITION OF DECOY RECEPTOR 3 MASTER CALIBRATOR	12
15. STABILITY OF SERUM / PLASMA SAMPLES AT 4°C.....	12
16. NORMAL VALUE AND NORMAL RANGE IN MOUSE SERUM.....	12
17. TROUBLESHOOTING AND FAQs	13
18. REFERENCES.....	14

Use only the actual version of Product Data Sheet enclosed with the kit!

1. Intended Use

The RD291036200 Mouse AFABP ELISA is a biotin labelled antibody based sandwich enzyme immunoassay for the quantitative measurement of Mouse AFABP in serum and plasma. It is intended for *research use only*.

Features

- The total assay time is less than four hours.
- The kit measures total serum or plasma AFABP.
- Quality Controls are mouse serum based. No animal sera are used.

2. Storage, Expiration

Store the kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

3. Summary

Protein definition:

Protein name: **Adipocyte FABP**

Synonyms:

aP2

AFABP

Fatty acid-binding protein, adipocyte (A-FABP)

Adipocyte lipid-binding protein (ALBP)

A-FABP

P2 adipocyte protein

Myelin P2 protein homolog

3T3-L1 lipid binding protein

422 protein

P15

Gene name:

FABP5

Ap2

Swissprot: P15090

NCBI / Protein: P15090

Adipocyte fatty acid binding protein AFABP is a 15 kDa member of the intracellular fatty acid binding protein (FABP) family, which is known for the ability to bind fatty acids and related compounds (bile acids or retinoids) in an internal cavity. AFABP is expressed in a differentiation-dependent fashion in adipocytes and is a critical gene in the regulation of the biological function of these cells. In mice, targeted mutations in FABP4 (gen also called: aP2 and its protein also called: P2 adipocyte protein, 3T3-L1 lipid binding protein) provide significant protection from hyperinsulinemia and insulin resistance in the context of both dietary and genetic obesity. Adipocytes obtained from AFABP-deficient mice also have reduced efficiency of lipolysis in vitro and in vivo, and these mice exhibited moderately improved systemic dyslipidemia. Recent studies also demonstrated AFABP expression in macrophages upon differentiation and activation. In these cells, AFABP modulates inflammatory responses and cholesterol ester accumulation, and total or macrophage-specific AFABP deficiency confers dramatic protection against atherosclerosis in the apoE^{-/-} mice. These results indicate a central role for AFABP in the development of major components of the metabolic syndrome through its distinct actions in adipocytes and macrophages.

Besides being active within the cell, AFAB appears to be a secreted protein. The extracellular role of secreted AFABP remains to be determined.

4. Test Principle

In the BioVendor's Mouse AFABP ELISA, calibrators or samples are incubated with a polyclonal anti-mouse AFABP antibody coated in microtiter wells. After two-hours incubation and a washing, biotin-labelled polyclonal anti-mouse AFABP antibody is added and incubated with captured AFABP for one hour. After a thorough wash, streptavidin-horseradish peroxidase conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate H₂O₂-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of AFABP. A standard curve is constructed by plotting absorbance values versus AFABP concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- For *research use only*.
- This kit contains components of mouse origin.
- Avoid contact with the acidic Stop Solution and Substrate Solution which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Reagents with different lot numbers should not be mixed.
- Reagents should not be used after the expiration specified on the kit label.

6. Reagents Supplied

<i>Kit Components</i>	<i>Quantity</i>
Microtiter Strips, coated with capture polyclonal Anti- mouse AFABP Antibody, sealed	96 wells
Biotin Labelled Anti- mouse AFABP Antibody, ready to use	13 ml
Streptavidin-Horseradish Peroxidase Conjugate, ready to use	13 ml
Mouse AFABP Master Calibrator, lyophilized, lyophilized	2 vials
Quality Control High, lyophilized	1 vial
Quality Control Low, lyophilized	1 vial
Dilution Buffer, ready to use	2x20 ml
Wash Solution Concentrate (10x)	100 ml
Substrate Solution (TMB), ready to use	13 ml
Stop Solution (0.2 M H ₂ SO ₄) ready to use	13 ml
Instruction Manual + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 100 µl
- Microplate reader with 450 nm filter
- Microplate shaker (optional)
- Software package facilitating data generation and analysis (optional)
- Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water

8. Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

Assay reagents are supplied ready-to-use, with the exception of Mouse AFABP Master Calibrator, Quality Controls and Wash Solution Concentrate (10x). Preparation of reagents for 1 plate:

- If you do not use the whole plate, return unused strips in the provided aluminium bag with dessicant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Preparation of reagents for 1 plate:

Wash Solution:

Dilute 100 ml of Wash Solution concentrate with 900 ml of deionized (distilled) water.

Stability and storage:

The diluted Wash Solution is stable for one month if stored at 2-8°C.

Mouse AFABP Calibrators:

Reconstitute Mouse AFABP Master Calibrator with 0.5 ml of Dilution Buffer. The concentration of the mouse AFABP in the stock solution is 50 ng/ml. Prepare Calibrators as follows:

<i>Calibrator volume</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
stock	-----	50 ng/ml
250 ul of stock	250 µl	25 ng/ml
250 ul of std. 25 ng/ml	250 µl	12.5 ng/ml
250 ul of std. 12.5 ng/ml	250 µl	6.25 ng/ml
250 ul of std. 6.25 ng/ml	250 µl	3.13 ng/ml
250 ul of std. 3.13 ng/ml	250 µl	1.56 ng/ml
250 ul of std. 1.56 ng/ml	250 µl	0.78 ng/ml

Prepared calibrators are ready to use, do not dilute them.

Stability and storage:

Calibrators are stable until the expiration date (see label on the box) when stored at -20°C.

Quality Controls:

Reconstitute each Quality Controls with 0.5 ml of Dilution Buffer (refer to the Certificate of Analysis for actual Quality Controls values).

Reconstituted Calibrators are ready to use, do not dilute them.

Stability and storage:

Reconstituted Quality Controls are stable until the expiration date (see label on the box) when stored at -20°C.

<i>Calibrator volume</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
stock	-----	14 U/ml
250 ul of stock	250 ul	7 U/ml
250 ul of std. 8 U/ml	250 ul	3.5 U/ml
250 ul of std. 4 U/ml	250 ul	1.75 U/ml
250 ul of std. 2 U/ml	250 ul	0.88 U/ml
250 ul of std. 1 U/ml	250 ul	0.44 U/ml

Rat AFABP Calibrators:

Reconstitute Rat AFABP Master Calibrator with 0.5 ml of Dilution Buffer. The concentration of the rat AFABP in the stock solution is 14 Units/ml. Prepare Calibrators as follows:

Prepared calibrators are ready to use, do not dilute them.

Stability and storage:

Calibrators are stable until the expiration date (see label on the box) when stored at -20°C.

9. Preparation of Samples

Dilute **mouse** serum samples prior to use 1:100 with Dilution Buffer, e.g. in two steps:

A/ 10 µl sample + 240 µl Dilution Buffer and mix well

B/ 100 µl from step A/ + 300 µl Dilution Buffer and mix well

Stability and storage:

See chapter 15.

Do not store the diluted (1:100) samples.

10. Assay Procedure

- 1) Pipet 100 µl of diluted Calibrators, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See Figure 1 for example of work sheet.
- 2) Incubate the plate at room temperature (ca. 25°C) for 2 hours, shaking at ca. 300 rpm on an orbital microplate shaker.
- 3) Wash the wells 5-times with Wash Solution (0.35 ml per well).
- 4) Pipet 100 µl of Biotin Labelled Anti-AFABP Antibody Solution into each well.
- 5) Incubate the plate at room temperature (ca. 25°C) for 1 hour, shaking at ca. 300 rpm on an orbital microplate shaker.
- 6) Wash the wells 5-times with Wash Solution (0.35 ml per well).
- 7) Pipet 100 µl of Streptavidin-HRP Conjugate.
- 8) Incubate the plate at room temperature (ca. 25°C) for 30 minutes, shaking at ca. 300 rpm on an orbital microplate shaker.
- 9) Wash the wells 5-times with Wash Solution (0.35 ml per well).
- 10) Pipet 100 µl of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
- 11) Incubate the plate for 10 minutes at room temperature. (The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C). No shaking!
- 12) Stop the colour development by adding 100 µl of Stop Solution.
- 13) Determine the absorbance by reading the plate at 450 nm. (The absorbance should be read within 5-15 minutes following step 12).

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine AFABP concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

	strip 1+ 2	strip 3 + 4	strip 5+ 6	strip 7+ 8	strip 9+10	strip 11+ 12
A	Calibrator 50	QC High	Sample 7	Sample 15	Sample 23	Sample 31
B	Calibrator 25	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
C	Calibrator 12,5	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	Calibrator 6,25	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	Calibrator 3,13	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	Calibrator 1,56	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	Calibrator 0,78	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37

Figure 1: Example of work sheet.

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The calibration curve is constructed by plotting the absorbance (Y) of calibrators versus *log* of the known concentration (X) of calibrators, using the four-parameter function.

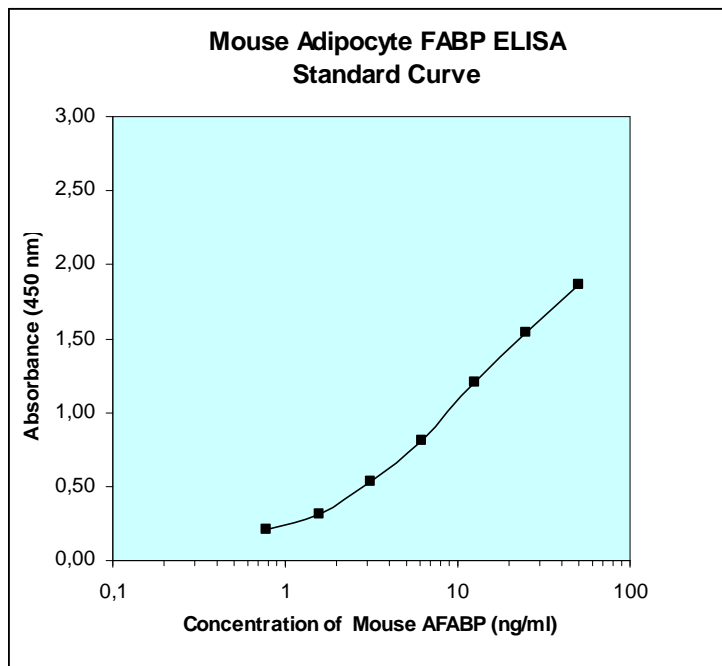


Figure 2: Standard Curve for mouse AFABP is plotted using the four-parameter function as a proportion of mouse AFABP concentration and absorbance at 450 nm.

As the Calibrators and the Quality Controls have not to be diluted, while the mouse samples 100-times, the values of samples calculated from the calibration curve have to be multiplied by a dilution factor of 100 to obtain the true results!

As the Calibrators and the Quality Controls have not to be diluted, while the rat samples 16-times, the values of samples calculated from the calibration curve have to be multiplied by a dilution factor of 16 to obtain the true results!

12. Limits of Assay

Results exceeding mouse AFABP level of 64 U/ml should be repeated with diluted samples. Dilution factors need to be taken into consideration in calculating the mouse AFABP concentration.

13. Performance Characteristics

- **Sensitivity**

The limit of detection (defined as such a concentration of mouse AFABP giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3 \times SD_{blank}$) is following:

Analytical Limit of Detection – using mouse AFABP values in wells is 0.31 ng/ml

Assay Sensitivity - the dilution factor have to be taken into the consideration if assaying diluted samples

Assay Sensitivity = Limit of Detection x dilution factor = 0.31ng/ml x 100 = 31ng/ml

*Dilution Buffer is pipetted into blank wells.

- **Specificity**

The antibodies in Mouse AFABP ELISA kit are highly specific for mouse and rat AFABP with no detectable crossreactivities to mouse/rat leptin, adiponectin, resistin at 50 ng/ml.

- **Precision**

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD	CV (%)
1	437	29.8	6.8
2	665	32.6	4.9

Inter-assay (Run-to-Run) (n=3)

Sample	Mean (ng/ml)	SD	CV (%)
1	463	40.8	8.8

2	711	49.1	6.9
---	-----	------	-----

- **Dilution Linearity**

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	679	-	-
	1:2	352	340	103.5
	1:4	186	170	109.6
2	-	445	-	-
	1:2	212	223	95.0
	1:4	116	111	105.0

14. Definition of AFABP Master Calibrator

A mouse serum Adipocyte Fatty Acid Binding Protein is used as the calibrator.

15. Stability of serum / plasma samples at 4°C

Samples should be stored at -20°C. However, no decline was observed in concentration of AFABP in serum and plasma samples when stored at 4°C for 2 weeks. To avoid microbial contamination add NaN₃ to a final concentration 0.1% to the samples.

16. Normal value and normal range in mouse serum

Group definition: mouse sera taken from 146 random selected BalbC, 2-10 months old
 Normal value (mean +/- SEM) = 672 +/- 37 ng/ml
 Normal range (mean +/- 2 SD) = 672 +/- 441 ng/ml

However, it is recommended that each laboratory establishes its own normal range of AFABP. The normal range / normal value depends on the tested mouse strain and should be therefore regarded as guideline only.

17. Troubleshooting and FAQs

1/ Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature

2/ High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time should be decreased before addition of Stop Solution

3/ High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing

4/ Drift

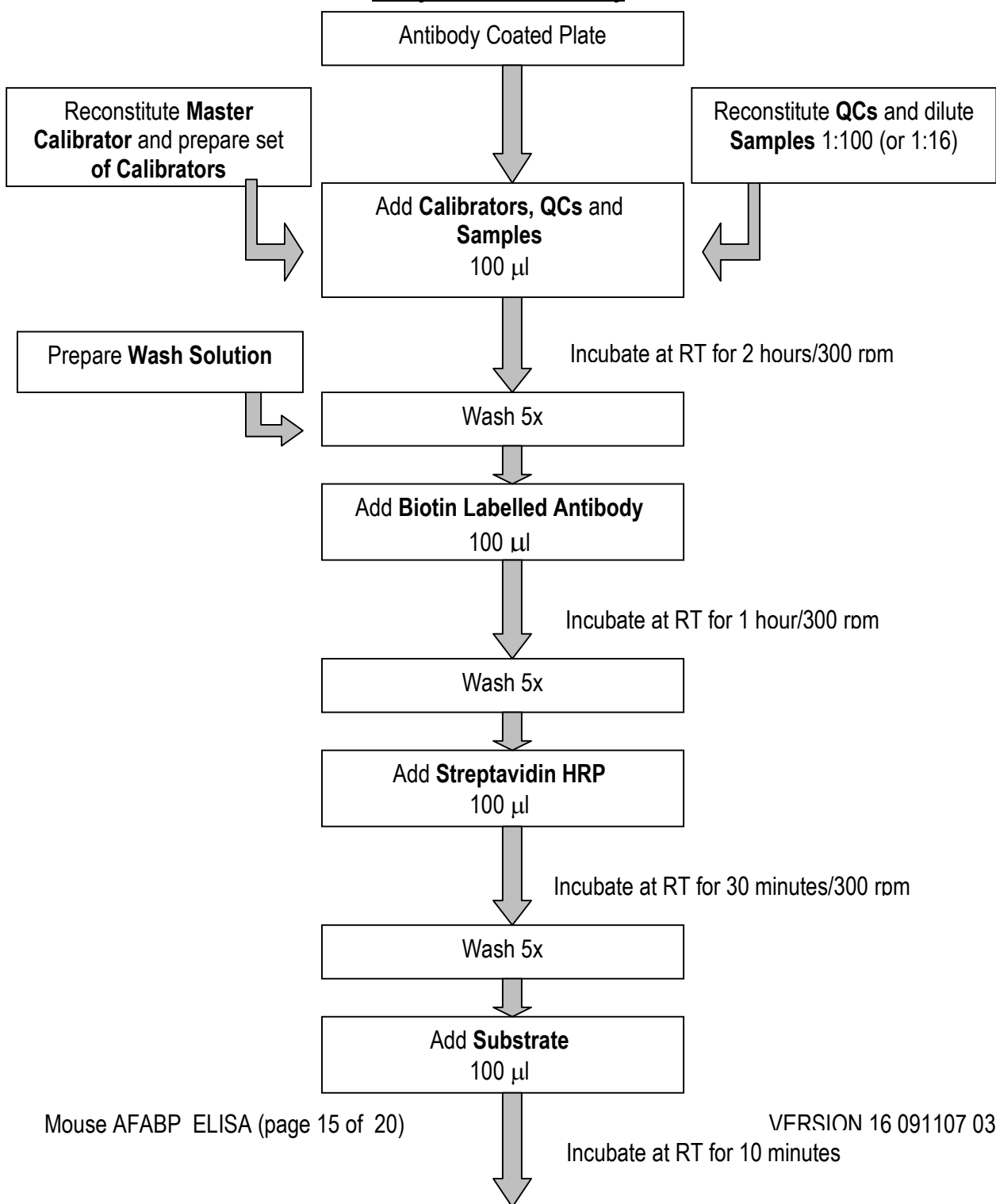
- Inadequate rpm of orbital shaker
- Inadequate rehydration volume for the calibrators and Quality Controls
- Insufficient mixing of reagents and samples before use
- Expiration date for the reagent exceeded

18. References

- Makowski L, Brittingham KC, Reynolds JM, Suttles J and Hotamisligil GS: The Fatty Acid-binding Protein, aP2, Coordinates Macrophage Cholesterol Trafficking and Inflammatory Activity. *J Biol Chem.* 2005 Apr 1;280(13):12888-95.
- Maeda K, Cao H, Kono K, Gorgun CZ, Furuhashi M, Uysal KT, Cao Q, Atsumi G, Malone H, Krishnan B, Minokoshi Y, Kahn BB, Parker RA and Hotamisligil GS: Adipocyte/macrophage fatty acid binding proteins control integrated metabolic responses in obesity and diabetes. *Cell Metabolism*, Volume 1, Issue 2, February 2005, Pages 107-119.
- Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Combined adipocyte-macrophage fatty acid-binding protein deficiency improves metabolism, atherosclerosis, and survival in apolipoprotein E-deficient mice. *Circulation.* 2004 Sep 14;110(11):1492-8.
- Lehmann F, Haile S, Axen E, Medina C, Uppenberg J, Svensson S, Lundback T, Rondahl L, Barf T: Discovery of inhibitors of human adipocyte fatty acid-binding protein, a potential type 2 diabetes target. *Bioorg Med Chem Lett.* 2004 Sep 6;14(17):4445-8.
- Damcott CM, Moffett SP, Feingold E, Barmada MM, Marshall JA, Hamman RF, Ferrell RE: Genetic variation in fatty acid-binding protein-4 and peroxisome proliferator-activated receptor gamma interactively influence insulin sensitivity and body composition in males. *Metabolism.* 2004 Mar;53(3):303-9.
- Jenkins-Kruchten AE, Bennaars-Eiden A, Ross JR, Shen WJ, Kraemer FB, Bernlohr DA: Fatty acid-binding protein-hormone-sensitive lipase interaction. Fatty acid dependence on binding. *J Biol Chem.* 2003 Nov 28;278(48):47636-43.
- Hertzfel AV, Bennaars-Eiden A, Bernlohr DA: Increased lipolysis in transgenic animals overexpressing the epithelial fatty acid binding protein in adipose cells. *J Lipid Res.* 2002 Dec;43(12):2105-11.
- Fu Y, Luo N, Lopes-Virella MF, Garvey WT: The adipocyte lipid binding protein (ALBP/aP2) gene facilitates foam cell formation in human THP-1 macrophages. *Atherosclerosis.* 2002 Dec;165(2):259-69.
- Storch J, Veerkamp JH, Hsu KT: Similar mechanisms of fatty acid transfer from human and rodent fatty acid-binding proteins to membranes: liver, intestine, heart muscle, and adipose tissue FABPs. *Mol Cell Biochem.* 2002 Oct;239(1-2):25-33.
- Fisher RM, Hoffstedt J, Hotamisligil GS, Thorne A, Ryden M: Effects of obesity and weight loss on the expression of proteins involved in fatty acid metabolism in human adipose tissue. *Int J Obes Relat Metab Disord.* 2002 Oct;26(10):1379-85.
- Boord JB, Maeda K, Makowski L, Babaev VR, Fazio S, Linton MF, Hotamisligil GS: Adipocyte fatty acid-binding protein, aP2, alters late atherosclerotic lesion formation in severe hypercholesterolemia. *Arterioscler Thromb Vasc Biol.* 2002 Oct 1;22(10):1686-91.

- Fisher RM, Eriksson P, Hoffstedt J, Hotamisligil GS, Thorne A, Ryden M, Hamsten A, Arner P: Fatty acid binding protein expression in different adipose tissue depots from lean and obese individuals. *Diabetologia*. 2001 Oct;44(10):1268-73.
- Scheja L, Makowski L, Uysal KT, Wiesbrock SM, Shimshek DR, Meyers DS, Morgan M, Parker RA, Hotamisligil GS: Altered insulin secretion associated with reduced lipolytic efficiency in aP2^{-/-} mice. *Diabetes*. 1999 Oct;48(10):1987-94.
- Coe NR, Simpson MA, Bernlohr DA: Targeted disruption of the adipocyte lipid-binding protein (aP2 protein) gene impairs fat cell lipolysis and increases cellular fatty acid levels. *J Lipid Res*. 1999 May;40(5):967-72.
- Baxa CA, Sha RS, Buelt MK, Smith AJ, Matarese V, Chinander LL, Boundy KL and Bernlohr DA: Human adipocyte lipid-binding protein: purification of the protein and cloning of its complementary DNA. *Biochemistry*. 1989; 28 (22), 8683-8690.

Assay Procedure Summary



12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
2									
1									
	A	B	C	D	E	F	G	H	

Notes:
