Quantikine® ELISA

Mouse/Rat FABP1/L-FABP Immunoassay

Catalog Number RFBP10

For the quantitative determination of mouse or rat Fatty Acid Binding Protein 1 (FABP1) concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Fatty Acid Binding Protein 1 (FABP1), also known as 14 kDa Selenium-binding protein, Z protein and FABP-L/Liver, is a 14 kDa member of the FABP family, calycin superfamily of molecules (1-3). It is a monomeric intracellular protein that contains selenium, but not as a component of the amino acid selenocysteine (4, 5). Mouse FABP1 is 127 amino acids (aa) in length (4). It possesses an initiating acetylated methionine, plus a lipocalin region (aa 4-126) that spans almost the entire length of the molecule. Structurally, FABP1 forms a 10-stranded β-barrel that interacts with a variety of molecules in a ligand-binding cavity. Notably, this cavity is large enough to accommodate two ligands simultaneously (a characteristic unique among FABPs), with each ligand adopting a different orientation (1, 3). There are also two N-terminal α-helices that interact with phospholipid-containing membranes. This interaction facilitates the delivery of different FABP1 ligands to distinct target sites (1). Full-length mouse FABP1 shares 94%, 84%, 83%, and 84% aa sequence identity with rat, human, porcine, and canine FABP1, respectively. Cells known to express FABP1 include hepatocytes (6, 7), simple columnar (mucosal) epithelium of the small intestine (2, 8), and proximal tubular epithelium of the kidney (at very low levels) (3, 9).

Functionally, FABP1 is known to bind and transport cholesterol, straight and branched long-chain fatty acids, heme, Vitamin K, and lysophospholipids (a phospholipid with only one rather than two fatty acid chains) (1, 6, 10). This can occur both extracellularly and intracellularly. Extracellular FABP1 is presumed to arise from the liver, possibly via a non-traditional secretory mechanism (9, 11). Its target appears to be megalin which is expressed on the luminal surface of proximal tubule epithelium. Once bound, it is internalized and directed into lysosomes. This may potentially either neutralize toxic lipids, or provide an energy source for the kidney (9). Within the cell, FABP1 is believed to act both in the nucleus and cytoplasm. FABP1 apparently enters the nucleus via a nuclear pore and delivers its cargo of fatty acids to PPARα, a nuclear factor that initiates the transcription of enzymes involved in the β-oxidation of fatty acids (12). In the cytoplasm, FABP1 circulates in the cytosol, generally directing its fatty acid cargo towards oxidative pathways (8). Through a peroxisome targeting sequence that is found in its last three amino acids, FABP1 will also enter peroxisomes and stimulate the activity of β-oxidizing enzymes. This creates fatty acids that can be transported out of the peroxisome via FABP1 (13). Finally, FABP1 is suggested to play a key role in the formation of prechylomicron vesicles by the ER. Potentially through FABP1 bound lysophospholipid, triacylglycerol and apolipoprotein-containing vesicles are induced to bud-off from the ER membrane on their way to the Golgi, providing a key first-step in chylomicron formation (14).

The Quantikine® Mouse/Rat FABP1/L-FABP-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure FABP1 in mouse or rat cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant rat FABP1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant rat FABP1. Results obtained using natural mouse or rat FABP1 showed dose response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse/rat FABP1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse/rat FABP1 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any FABP1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat FABP1 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of FABP1 bound in the initial step. The sample values are then read off the standard curve.

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.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse/Rat FABP1 Microplate	894220	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse/rat FABP1.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat FABP1 Conjugate	894221	12 mL of a polyclonal antibody specific for mouse/rat FABP1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat FABP1 Standard	894222	Recombinant rat FABP1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat FABP1 Control	894223	Recombinant rat FABP1 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-17	895433	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed, icteric, and lipemic samples are not suitable for use in this assay.

Urine - Collect urine using a metabolic cage. Remove any particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

SAMPLE PREPARATION

Mouse serum and plasma samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Rat serum and plasma samples require at least a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Rat urine samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

* See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse/Rat FABP1 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

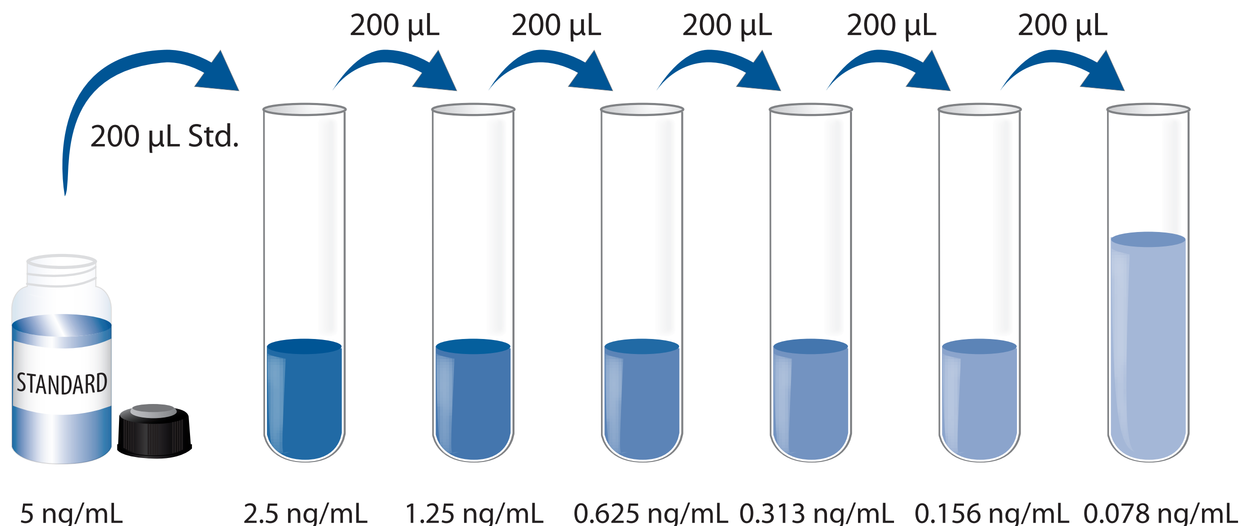
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 μL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse/Rat FABP1 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Mouse/Rat FABP1 Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 5 ng/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 μL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse/Rat FABP1 Standard (5.0 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 μL of Assay Diluent RD1-17 to each well.
4. Add 50 μL of standard, control, or sample* per well. Gently tap the plate to ensure thorough mixing. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record the standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 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 Mouse/Rat FABP1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 μL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 μL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, 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 the Sample Preparation section.

CALCULATION OF RESULTS

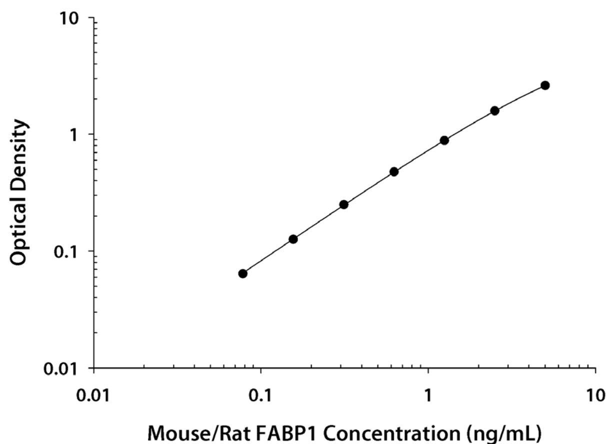
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse/rat FABP1 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.

TYPICAL DATA

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



(ng/mL)	O.D.	Average	Corrected
0	0.028 0.029	0.028	—
0.078	0.091 0.092	0.092	0.064
0.156	0.153 0.155	0.154	0.126
0.313	0.274 0.283	0.278	0.250
0.625	0.503 0.504	0.504	0.476
1.25	0.908 0.912	0.910	0.882
2.5	1.602 1.617	1.610	1.582
5	2.614 2.656	2.635	2.607

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	0.28	0.69	1.22	0.27	0.68	1.25
Standard deviation	0.01	0.02	0.03	0.01	0.03	0.06
CV (%)	3.6	2.9	2.5	3.7	4.4	4.8

RECOVERY

The recovery of mouse/rat FABP1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	95	90-100%
Mouse urine* (n=4)	96	86-108%
Rat serum* (n=4)	100	87-116%
Rat EDTA plasma* (n=4)	94	84-107%
Rat heparin plasma* (n=4)	100	92-111%
Rat urine* (n=4)	99	95-105%

*Samples were diluted prior to assay as described in the Sample Preparation section.

SENSITIVITY

Sixty-seven assays were evaluated and the minimum detectable dose (MDD) of mouse/rat FABP1 ranged from 0.001-0.029 ng/mL. The mean MDD was 0.005 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant rat FABP1 produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse/rat FABP1 in each matrix were diluted with calibrator diluent and assayed.

Mouse Samples		Cell culture supernates* (n=4)	Serum * (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine (n=4)
1:2	Average % of Expected	103	102	103	102	112
	Range (%)	96-110	97-105	102-105	99-106	109-115
1:4	Average % of Expected	104	106	107	104	113
	Range (%)	95-111	104-108	98-118	95-113	111-114
1:8	Average % of Expected	104	108	111	104	114
	Range (%)	90-120	100-118	104-118	102-108	106-118
1:16	Average % of Expected	95	111	110	112	111
	Range (%)	82-112	102-119	102-116	105-119	107-112

Rat Samples		Cell culture supernates* (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)	Urine* (n=4)
1:2	Average % of Expected	103	104	104	105	101
	Range (%)	100-109	102-106	102-108	102-110	99-105
1:4	Average % of Expected	105	110	108	106	105
	Range (%)	101-110	110-111	98-114	102-109	103-106
1:8	Average % of Expected	104	112	106	109	104
	Range (%)	100-108	110-113	97-116	100-117	100-107
1:16	Average % of Expected	100	114	108	109	——
	Range (%)	95-108	110-119	98-116	99-116	——

*Samples were diluted prior to assay.

SAMPLE VALUES

Serum/Plasma - Mouse and rat samples were evaluated for the presence of mouse/rat FABP1 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Mouse serum (n=20)	6068	11.2-42,464	11,605
Mouse EDTA plasma (n=11)	301	8.40-1344	407
Mouse heparin plasma (n=11)	446	7.40-1538	502
Rat serum (n=20)	32.7	7.20-195	40.6
Rat EDTA plasma (n=10)	61.5	7.72-265	89.7
Rat heparin plasma (n=10)	201	13.0-948	304
Rat urine (n=16)	2.62	0.09-28	6.88

Sample Type	Mean of Detectable (ng/mL)	% Detectable	Range (ng/mL)
Mouse urine (n=15)	0.135	20	ND-0.364

ND=Non-detectable

Cell Culture Supernates:

Livers from mice were removed, rinsed in PBS, and kept on ice. The livers were then homogenized using a tissue homogenizer and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. An aliquot of the cell culture supernate was removed and assayed for mouse/rat FABP1, and measured 46,320 ng/mL.

Livers from rats were removed, rinsed in PBS, and kept on ice. The livers were then homogenized using a tissue homogenizer and cultured in DME supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 50 µg/mL gentamicin solution for 3 days. An aliquot of the cell culture supernate was removed and assayed for mouse/rat FABP1, and measured 36,920 ng/mL.

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SPECIFICITY

This assay recognizes natural and recombinant mouse and rat FABP1.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse/rat FABP1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Cystatin C
FABP4
FABP5
TIM-1/KIM-1/HAVCR

Recombinant rat:

Cystatin C
Lipocalin-2/NGAL
TIM-1/KIM-1/HAVCR

Recombinant human:

Cystatin C
FABP2
FABP3
FABP4
FABP5
FABP7
FABP8
FABP9

Recombinant human FABP1 does not interefere but does cross-react approximately 2% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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

NOTES

NOTES

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