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ab222875 Human FABP-1 ELISA Kit

For the quantitative measurement of human FABP-1 in plasma, serum, saliva, milk, cerebral spinal fluid and cell lysate, and tissue samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Human FABP-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit (ab222875) is designed for the quantitative measurement of human FABP-1 protein in plasma, serum, saliva, milk, cerebral spinal fluid and cell lysate, and tissue samples.

The assay employs a quantitative sandwich enzyme immunoassay technique that measures human FABP-1 in approximately 4 hours. A polyclonal antibody specific for human FABP-1 has been pre-coated onto a 96-well microplate with removable strips. FABP-1 in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for human FABP-1, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Mitochondrial glutamate oxaloacetate transaminase 2 (FABP-1), also called mitochondrial aspartate aminotransferase (mAspAT) and fatty acid-binding protein, belongs to the class-I pyridoxal-phosphate-dependent aminotransferase family. GOT is a pyridoxal phosphate-dependent enzyme, which exists in cytoplasmic GOT1 and inner-membrane mitochondrial FABP-1 forms. GOT plays a role in amino acid metabolism and in the urea and tricarboxylic acid cycles. The two enzymes show close homology. FABP-1 is a dimer containing two identical subunits, each 45 kDa with about 401 amino acids. FABP-1 acetylation stimulates the malate–aspartate NADH shuttle activity and oxidative protection. It is also involved in cell proliferation and energy production.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed



Add 50 μ L standard or sample to appropriate wells and incubate for 2 hours



Wash wells. Add 50 μ L Biotinylated Antibody to wells and incubate for 1 hour



Wash wells. Add 50 μ L Streptavidin-Peroxidase Complex to wells and incubate for 30 minutes



Wash wells. Add 50 μ L Chromogen Substrate to wells and incubate for 30 minutes



Add 50 μ L Stop Solution and **immediately** read OD at 450 nm

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt, apart from Human FABP-1 Standard, Biotinylated Human FABP-1 and 100X Streptavidin-Peroxidase Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti- Human FABP-1 coated Microplate (12 x 8 wells)	96 wells	+4°C
Human FABP-1 Standard	2 x 1 vial	-20°C
Biotinylated Human FABP-1	1 vial	-20°C
10X Diluent N Concentrate	1 x 30 mL	+4°C
1X Standard Diluent	1 x 2 mL	+4°C
20X Wash Buffer Concentrate	2 x 30 mL	+4°C
100X Steptavidin-Peroxidase Conjugate	1 x 80 µL	-20°C
Chromogen Substrate	1 x 7 mL	+4°C
Stop Solution	1 x 11 mL	+4°C
Sealing Tapes	3 units	+4°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at 450.
- Deionized water.
- Multi- and single-channel pipettes.
- Tubes for standard dilution.

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 10X Diluent N Concentrate (30 mL):

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Diluent N by diluting 10X Diluent N 10-fold with deionized water to produce a 1X solution. Store for up to 30 days at +4°C.

9.2 20X Wash Buffer Concentrate (2 x 30 mL):

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Prepare 1X Wash Buffer by diluting 20X Wash Buffer 20-fold with deionized water to produce a 1X solution. Mix thoroughly and gently.

9.3 Biotinylated Human FABP-1 (120 µL):

Briefly spin down the Biotinylated Human FABP-1 and dilute the desired amount 30-fold with Diluent N to produce a 1X solution. Any remaining solution should be frozen at -20°C.

9.4 100X Streptavidin-Peroxidase Conjugate (80 µL):

Briefly spin down the 100X Streptavidin-Peroxidase Conjugate and dilute the desired amount 100-fold with Diluent N to produce a 1X solution. Any remaining solution should be frozen at -20°C.

9.5 Anti-Human FABP-1 coated Microplate (12 x 8 wells):

Ready to use. Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

9.6 Chromagen Substrate (8 mL):

Ready to use. Store at +4°C.

9.7 Sealing Tapes (3 units):

Ready to use. Store at +4°C.

9.8 Stop Solution (12 mL):

Ready to use. Store at +4°C.

9.9 Human FABP-1 Standard (140 ng, 2 vials, lyophilized):

Reconstitute the Human FABP-1 Standard (140 ng) with 0.7 ml of Standard Diluent to generate a 200 ng/mL standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Reconstitute the FABP-1 Stock to generate an 200 ng/mL Standard #1.

- 10.1.1 First consult the FABP-1 Standard vial to determine the mass of protein in the vial.
- 10.1.2 Calculate the appropriate volume of 1X Diluent N to add when resuspending the FABP-1 Standard vial to produce an 200 ng/mL FABP-1 Standard stock by using the following equation:

C_S = Starting mass of FABP-1 Standard stock (see vial label) (ng)

C_F = 200 ng/mL FABP-1 Standard #1 final required concentration

V_D = Required volume of 1X Diluent N for reconstitution (μ L)

Calculate total required volume 1X Diluent N for resuspension:

$$(C_S / C_F) * 1,000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 140 ng of FABP-1 Standard in vial

C_F = 200 ng/mL FABP-1 Standard #1 final concentration

V_D = Required volume of 1X Diluent N for reconstitution

$(140 \text{ ng} / 200 \text{ ng/mL}) * 1,000 = 700 \mu\text{L}$

- 10.1.3 First briefly centrifuge the FABP-1 Standard Vial to collect the contents on the bottom of the tube.
- 10.1.4 Reconstitute the FABP-1 Standard vial by adding the appropriate calculated amount VD of 1X Diluent N to the vial to generate the 200 ng/mL FABP-1 **Standard #1**. Mix gently and thoroughly.
- 10.2 Allow the reconstituted 200 ng/mL FABP-1 **Standard #1** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions.
- 10.3 From the standard stock solution (200 ng/ml), dilute 4-fold with 1X Diluent N to produce a 50 ng/ml standard working solution.
- 10.4 Label seven tubes #2 – 8.
- 10.5 Prepare duplicate or triplicate standard points by serially diluting the Standard Stock Solution (50 ng/mL) 1:2 with Diluent N to produce 25, 12.5, 6.25, 3.125 and 1.563 ng/mL solutions. 1X Diluent N serves as the zero standard (0 ng/mL).
- 10.6 Add 120 µL of 1X Diluent N to tube #2 – 8.
- 10.7 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.
- 10.8 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.
- 10.9 Using the table below as a guide, prepare subsequent serial dilutions.

Standard #	Volume to dilute (µL)	Volume Diluent N (µL)	FABP-1 (ng/mL)
1	Step 10.3		50
2	120 µL Standard #1	120	25
3	120 µL Standard #2	120	12.5
4	120 µL Standard #3	120	6.25
5	120 µL Standard #4	120	3.125
6	120 µL Standard #5	120	1.563
7	(Blank)	120	0

11. Sample Preparation

11.1 Plasma:

Collect plasma using one-tenth volume 0.1 M sodium citrate (EDTA or heparin can also be used as anti-coagulant). Centrifuge samples at 3,000 x g for 10 minutes and collect the plasma. A 2-fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.2 Serum:

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 x g for 10 minutes and remove serum. A 2-fold sample dilution is suggested into Diluent N; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.3 Cell Lysates:

Rinse cell with cold PBS and then scrape the cell into a tube with 5 ml of cold PBS and 0.5 M EDTA. Centrifuge suspension at 1500 rpm for 10 minutes at 4°C and aspirate supernatant. Resuspend pellet in ice-cold Lysis Buffer (PBS, 1% Triton X-100, protease inhibitor cocktail). For every 1×10^6 cells, add approximately 100 µl of ice-cold Lysis Buffer. Incubate on ice for 60 minutes. Centrifuge at 13000 rpm for 30 minutes at 4°C and collect supernatant. If necessary, dilute samples into MIX Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

11.4 Tissue:

Extract tissue samples with 0.1 M phosphate-buffered saline (pH 7.4) containing 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. If necessary, dilute samples into MIX Diluent; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

11.5 Saliva:

Collect saliva using sample pot. Centrifuge samples at 800 x g for 10 minutes. A 2-fold sample dilution is suggested into Diluent N;

however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.6 Milk:

Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes and as say. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

11.7 Cerebral spinal fluid:

Collect cerebral spinal fluid (CSF) using sample pot. Centrifuge samples at 3000 x g for 10 minutes. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
 - We recommend that you assay all standards, controls and samples in duplicate.
 - Prepare all reagents, working standards, and samples as directed in the previous sections.
- 12.1 Prepare all reagents, working standards, and samples as directed in the previous sections.
 - 12.2 Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
 - 12.3 Add 50 µL of sample or standard to appropriate wells. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
 - 12.4 Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
 - 12.5 Add 50 µL 1X Biotinylated Human FABP-1 to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
 - 12.6 Wash the microplate as described above (Step 12.4).
 - 12.7 Add 50 µL of 1X Streptavidin-Peroxidase Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
 - 12.8 Wash the microplate as described above (Step 12.4).
 - 12.9 Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops.

- 12.10** Add 50 μ L of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
- 12.11** **Immediately**, read the absorbance on a microplate reader at a wavelength of 450 nm. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only.

Δ Note: Some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

13. Calculations

- 13.1 Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- 13.2 To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using four-parameter or log-log logistic curve-fit.
- 13.3 Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

14. Typical Data

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

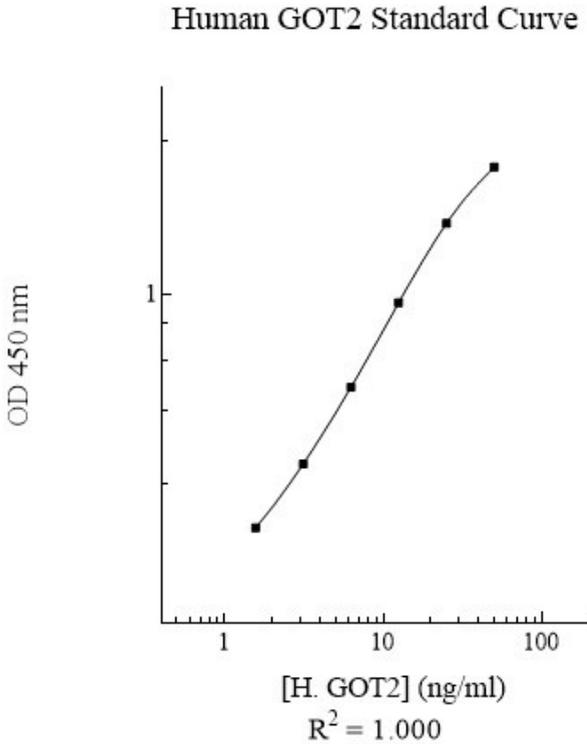


Figure 1. Example of human GOT 2 standard curve in Diluent N. The FABP-1 standard curve was prepared as described in Section 10. Background-subtracted data are graphed.

Standard Curve Measurements			
Concentration (ng/mL)	O.D 450 nm		Mean O.D
	1	2	
50	2.381	2.307	2.344
25	1.635	1.577	1.606
12.5	0.921	0.967	0.944
6.25	0.519	0.551	0.535
3.125	0.308	0.332	0.320
1.563	0.201	0.215	0.208
0	0.084	0.09	0.087

15. Typical Sample Values

SENSITIVITY –

The calculated minimal detectable dose (MDD) is 0.66 ng/mL. The MDD was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

PRECISION –

Intra-assay and inter-assay coefficients of variation were 4.9% and 9.4%, respectively.

	Intra-Assay Precision	Inter-Assay Precision
Average CV (%)	4.7	9.3

RECOVERY –

Standard Added Value	6.25 – 25 ng/mL
Recovery %	89 – 114%
Average Recovery %	97%

16. Assay Specificity

This kit recognizes both native and recombinant human FABP-1 protein in serum, plasma, saliva, milk, cerebral spinal fluid, cell lysate and tissue samples.

CROSS REACTIVITY

No significant cross-reactivity observed with GOT1, FABP2, FABP3, FABP4, FABP5, FABP6, FABP7, FABP8, FABP9, and FAAH2.

17. Species Reactivity

This kit recognizes human FABP-1 protein.

Species	Cross Reactivity (%)
Dog	10
Cow	None
Monkey	70
Mouse	35
Rat	40
Plg	50
Rabbit	None

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are dry after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing

Unexpectedly Low or High Signal Intensity	Microplate was left unattended between steps	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.
Deficient Standard Curve fit	Non-optimal sample dilution	Sandwich ELISA: If samples generate OD values higher than the highest standard point (P1), dilute samples further and repeat the assay. User should determine the optimal dilution factor for samples.
	Contamination of reagents.	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

19. Notes

Technical Support

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