Distribuito in ITALIA da
Li StarFish S.r.l.
Via Cavour, 35
20063 Cernusco S/N (MI)
telefono 02-92150794
fax 02-92157285
info@listarfish.it
www.listarfish.it

Manual

IDK® FABP2 ELISA

For the in vitro determination of fatty acid binding protein 2 (FABP2) in serum and plasma

Valid from 2019-09-16

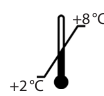


Table of Contents

1. **INTENDED USE** 15
2. **INTRODUCTION** 15
3. **MATERIAL SUPPLIED** 15
4. **MATERIAL REQUIRED BUT NOT SUPPLIED** 16
5. **STORAGE AND PREPARATION OF REAGENTS** 16
6. **STORAGE AND PREPARATION OF SAMPLES** 17
   Sample storage 17
7. **ASSAY PROCEDURE** 17
   Principle of the test 17
   Test procedure 18
8. **RESULTS** 19
9. **LIMITATIONS** 20
10. **QUALITY CONTROL** 20
   Reference range 20
11. **PERFORMANCE CHARACTERISTICS** 21
   Accuracy – Precision 21
   Linearity 21
   Analytical Sensitivity 22
12. **PRECAUTIONS** 22
13. **TECHNICAL HINTS** 23
14. **GENERAL NOTES ON THE TEST AND TEST PROCEDURE** 23
15. **REFERENCES** 24
   General literature 24

1. INTENDED USE

This enzyme immunoassay is intended for the quantitative determination of fatty acid binding protein 2 (FABP2) in serum and plasma. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

The intracellular fatty acid-binding protein (FABPs) family consists of nearly 20 members. It is divided in three subgroups: the hepatic, intestinal and cardiac group. They participate in the uptake, intracellular metabolism and/or transport of long-chain fatty acids. They may also be responsible in the modulation of cell growth and proliferation.

FABP2 is probably involved in triglyceride-rich lipoprotein synthesis. It binds saturated long-chain fatty acids with a high affinity, but binds with a lower affinity to unsaturated long-chain fatty acids. FABP2 may also help maintain energy homeostasis by functioning as a lipid sensor. It is an abundant cytosolic protein in small intestine epithelial cells.

Possible research areas

- pancreatitis [1, 2]
- necrotising enterocolitis [3]
- Celiac disease [4]
- Crohn's disease [5]

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR6809	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	1 x 100 ml
KR6809	2. AB	Detection antibody, ready-to-use	1 x 11 ml
KR6809	CONJ	Conjugate, ready-to-use	1 x 11 ml
KR6809	STD	Standards, lyophilised (0; 111; 333; 1000; 3000 pg/ml)	2 x 5 vials
KR6809	CTRL1	Control, lyophilised (see specification for range)	2 x 1 vial

Cat. No.	Label	Kit components	Quantity
KR6809	CTRL2	Control, lyophilised (see specification for range)	2 x 1 vial
KR6809	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 50 ml
KR0002.15	SUB	Substrate (tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water*
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of ultrapure water (water type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is

stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.

- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. Before use, the STD and CTRL have to be reconstituted with **500 µl of ultrapure water** and mixed by gentle inversion to ensure complete reconstitution. Allow the vial content to dissolve for 10 minutes and then mix thoroughly. **Standards and controls** (reconstituted STD and CTRL) **can be stored at 2–8 °C for 4 weeks.**
- All other test reagents are ready-to-use. Test reagents are stable until the expiry date (see label) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF SAMPLES

Sample storage

Freshly collected EDTA plasma or serum can be stored at -20 °C.

Serum/plasma samples

EDTA plasma or serum samples must be diluted **1:5** before performing the assay,

e. g. **50 µl** sample + **200 µl** sample dilution buffer (SAMPLEBUF), mix well.

100 µl of the dilution are used in each well of the test.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of FABP2 in serum and plasma samples.

Standards and prepared samples to be analysed for FABP2 are pipetted into the wells of a microtiter plate coated with an anti-FABP2 antibody. In this first incubation step, FABP2 from the samples ist bound to the primary antibody coated to the microtiter plate. Then, a biotin-labelled anti-FABP2 antibody is added. The next step is adding the streptavidin-peroxidase conjugate. We now have the follwing complex at the wall of the microtiter well:

Primary antibody – FABP2 – biotinylated antibody – streptavidin-peroxidase conjugate

Tetramethylbenzidine (TMB) is used as peroxidase substrate. The enzymatic reaction is stopped by adding acid, causing a colour change from blue to yellow. The resulting

chomogenic compound is measured photometrically at 450 nm. The intensity of the colour is directly proportinal to the FABP2 concentration.

A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. FABP2, present in the samples, is determined directly from this curve.

Test procedure

Before use, bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Mark the positions of standards/controls/samples on a protocol sheet.

Take as many microtiter strips as needed from the kit. Store unused strips together with the desiccant bag in the closed aluminium packaging at 2–8 °C. Strips are stable until expiry date stated on the label.

For automated ELISA processors, the given protocol may need to be adjusted according to the specific features of the respective automated platform. For further details please contact your supplier or Immundiagnostik AG.

We recommend to carry out the tests in duplicate.

1.	**Before use**, wash the wells **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each **100 µl standards/controls/diluted samples** into the respective wells.
3.	Cover the strips and incubate for **1 hour** at room temperature (15–30 °C) on a **horizontal shaker***.
4.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add **100 µl detection antibody** (2. AB) into each well.
6.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a **horizontal shaker***.
7.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add **100 µl conjugate** (CONJ) into each well.

9.	Cover the strips and incubate for **30 min** at room temperature (15–30 °C) on a **horizontal shaker***.
10.	Discard the content of each well and wash **5 times** with **250 µl wash buffer**. After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add **100 µl substrate** (SUB) into each well.
12.	Incubate for **10–20 min**** at room temperature (15–30 °C) in the **dark**.
13.	Add **100 µl stop solution** (STOP) into each well and mix well.
14.	Determine **absorption immediately** with an ELISA reader at **450 nm** against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at **405 nm** against 620 nm (or 690 nm) as a reference.

* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

** The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the 4 parameter algorithm.

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. Point-to-point calculation

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. Spline algorithm

We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum/plasma samples

The obtained results have to be multiplied by the **dilution factor of 5** to get the actual concentrations.

In case **another dilution factor** has been used, multiply the obtained result by the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × sample dilution factor to be used

The lower limit of the measurement range can be calculated as:

LoB × sample dilution factor to be used

LoB see chapter "Performance Characteristics".

10. QUALITY CONTROL

Immundiagnostik AG recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n=23

The repeatability was assessed with 2 serum samples under constant parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [pg/ml]	CV [%]
1	3881.68	5.6
2	1038.15	5.1

Reproducibility (Inter-Assay); n=36

The reproducibility was assessed with 4 serum samples under varying parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [pg/ml]	CV [%]
1	1176.59	13.1
2	1748.64	13.3
3	591.97	13.6
4	2346.05	11.4

Linearity

The linearity states the ability of a method to provide results proportional to the concentration of analyte in the test sample within a given range. This was assessed according to CLSI guideline EP6-A by serial dilution of 3 different serum samples.

For FABP2 in serum and plasma, the method has been demonstrated to be linear from 176.44 to 2778.30 pg/ml, showing a non-linear behaviour of less than ±20 % in this interval.

Sample	Dilution	Expected [pg/ml]	Obtained [pg/ml]	Recovery [%]
1	undiluted	-	2778.30	-
	1:2	1389.15	1574.24	113.32
	1:3	926.10	992.56	107.18
	1:4	694.58	734.94	105.81
	1:5	555.66	555.41	99.95
2	1:3	-	529.32	-
	1:4	396.99	397.41	100.11
	1:5	317.59	359.86	113.31
	1:6	264.66	307.05	116.02
	1:7	226.85	270.53	119.26
	1:8	198.49	223.65	112.67
	1:9	176.44	220.49	124.96
3	1:3	-	643.86	-
	1:4	482.89	478.20	99.03
	1:5	386.32	425.49	110.14
	1:6	321.93	381.11	118.38
	1:7	275.94	305.25	110.62
	1:8	241.45	291.27	120.64
	1:9	214.62	257.91	120.17

Analytical Sensitivity

Limit of blank, LoB 28.45 pg/ml

12. PRECAUTIONS

- All reagents in the kit package are for research use only.
- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.

- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic colour reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid (H_2SO_4), a strong acid. Although diluted, it still should be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analysed with each run.
- Reagents should not be used beyond the expiration date stated on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- Do not mix plugs and caps from different reagents.
- The assay should always be performed according to the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- *IDK*® is a trademark of Immundiagnostik AG.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

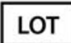
1. Kupčinskas, Juozas, Rolandas Gedgaudas, Hannes Hartman, Tomi Sippola, Outi Lindström, Colin Johnson, and Sara Regnér. 2018. "Intestinal Fatty Acid Binding Protein as a Marker of Necrosis and Severity in Acute Pancreatitis." *Pancreas* **47** (6): 1.
2. Goswami, Pooja, Ujjwal Sonika, Praneeth Moka, Vishnubhatla Sreenivas, and Anoop Saraya. 2017. "Intestinal Fatty Acid Binding Protein and Citrulline as Markers of Gut Injury and Prognosis in Patients With Acute Pancreatitis." *Pancreas* **46** (10): 1275–80.
3. Cheng, Shupeng, Jialin Yu, Min Zhou, Yan Tu, and Qi Lu. 2015. "Serologic Intestinal-Fatty Acid Binding Protein in Necrotizing Enterocolitis Diagnosis: A Meta-Analysis." *BioMed Research International* **2015**: 1–8.
4. Gross, Sascha, Marlou P M Adriaanse, Petula Nijeboer, Greetje J Tack, Ingrid M W van Hoogstraten, Gerd Bouma, Chris J Mulder, B Mary E von Blomberg, Anita C E Vreugdenhil, and Hetty J Bontkes. 2015. "Serum Intestinal-Fatty Acid Binding Protein as a Biomarker for Refractory Celiac Disease." *Journal of Gastrointestinal and Liver Diseases : JGLD* **24** (2): 258–59.
5. Sarikaya, Murat, Bilal Ergül, Zeynal Doğan, Levent Filik, Murat Can, and Latife Arslan. 2015. "Intestinal Fatty Acid Binding Protein (I-FABP) as a Promising Test for Crohn's Disease: A Preliminary Study." *Clinical Laboratory* **61** (1–2): 87–91.

Used symbols:

Temperature limitation

For research use only

Manufacturer



Lot number

Attention

Consult specification data sheet

Catalogue Number

To be used with

Contains sufficient for <n> tests

Use by

Consult instructions for use