**Human Intestinal FABP (FABP2) ELISA**

Product Data Sheet

Cat. No.: RD191246200R

For Research Use Only
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This kit is manufactured by:
BioVendor – Laboratorní medicína a.s.

Use only the current version of Product Data Sheet enclosed with the kit!
1. **INTENDED USE**

The RD191246200R Human Intestinal FABP (FABP2) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human intestinal FABP (I-FABP).

**Features**

- **It is intended for research use only**
- The assay time is less than 3.5 hours
- The kit measures I-FABP in serum, plasma (EDTA, citrate, heparin) and urine
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. **STORAGE, EXPIRATION**

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

For stability of opened reagents see Chapter 9.
3. INTRODUCTION

Intestinal fatty acid binding protein (I-FABP) or FABP2, named after the tissue in which it was first identified, is one of the nine different FABPs characterized in mammals. The fatty acid binding proteins (FABPs) are 15 kDa cytoplasmic proteins, members of the superfamily of small intracellular lipid-binding proteins (LBPs). The primary function of all FABPs is regulation of long-chain fatty acid uptake and intracellular transport.[1]

I-FABP is a cytosolic protein specifically present in mature enterocytes at the tip of the villus of small intestine and constitutes 2% of enterocyte proteins. I-FABP has high affinity for saturated and unsaturated long-chain fatty acids, and is believed to be involved in absorption and transport of dietary fatty acids. I-FABP has two forms: alanine-containing (A54) or threonine-containing (T54) protein which display differences in binding and transporting fatty acid across cells. The I-FABP genetic polymorphism Ala54Thr was found to be associated with increased fat oxidation, insulin resistance, elevated serum leptin levels, obesity and dyslipidemia in several populations, providing additional support for I-FABP role in energy metabolism and metabolic syndrome.[2,3,5]

Serum concentration of I-FABP is low in healthy subjects, but upon enterocyte membrane integrity loss, I-FABP is released in the circulation and its serum level is rapidly increased. This makes I-FABP useful as a plasma marker for enterocyte damage and indeed, I-FABP has been promoted as a promising new serological marker for intestinal disease. Several studies have demonstrated the use of I-FABP for early detection of intestinal injury such as necrotizing enterocolitis, acute intestinal ischemia, gut wall integrity loss in viral gastroenteritis, small bowel obstruction or Crohn’s disease.[4,6,7,8,10]

Thanks to its small size, I-FABP can quickly pass through the kidney to urine, which also gives an opportunity to measure it noninvasively in urine. A recent study found serum and urinary I-FABP levels are correlated with the length of resected bowel in infants with surgical treatment of necrotizing enterocolitis.[9] Urinary I-FABP can also distinguish necrotizing enterocolitis from sepsis in early stage of the disease.[11]

Areas of investigation:
Intestinal disease
4. TEST PRINCIPLE

In the BioVendor Human I-FABP ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human I-FABP antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human I-FABP antibody is added and incubated for 60 minutes with captured I-FABP. After another washing, streptavidin-HRP conjugate is added. After 30 minutes incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of I-FABP. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- For professional use only
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit may contain components of human or animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not been mixed thoroughly with the Substrate Solution.

Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements.

7. REAGENT SUPPLIED

<table>
<thead>
<tr>
<th>Kit Components</th>
<th>State</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody Coated Microtiter Strips</td>
<td>ready to use</td>
<td>96 wells</td>
</tr>
<tr>
<td>Biotin Labelled Antibody Conc. (100x)</td>
<td>concentrated</td>
<td>0.13 ml</td>
</tr>
<tr>
<td>Streptavidin-HRP Conjugate</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Master Standard</td>
<td>lyophilized</td>
<td>2 vials</td>
</tr>
<tr>
<td>Dilution Buffer</td>
<td>ready to use</td>
<td>2x 20 ml</td>
</tr>
<tr>
<td>Wash Solution Conc. (10x)</td>
<td>concentrated</td>
<td>100 ml</td>
</tr>
<tr>
<td>Substrate Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>ready to use</td>
<td>13 ml</td>
</tr>
<tr>
<td>Product Data Sheet + Certificate of Analysis</td>
<td>-</td>
<td>1 pc</td>
</tr>
</tbody>
</table>

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precise pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of shaking at approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)
9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use

Always prepare only the appropriate quantity of reagents for your test

Do not use components after the expiration date marked on their label

- Assay reagents supplied ready to use:

**Antibody Coated Microtiter Strips**

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

**Streptavidin-HRP Conjugate**

**Dilution Buffer**

**Substrate Solution**

**Stop Solution**

Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

- Assay reagents supplied concentrated or lyophilized:

**Human I-FABP Master Standard**

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!

Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). The resulting concentration of human I-FABP in the stock solution is 1280 pg/ml.

Prepare set of standards using Dilution Buffer as follows:

<table>
<thead>
<tr>
<th>Volume of Standard</th>
<th>Dilution Buffer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stock</td>
<td>-</td>
<td>1280 pg/ml</td>
</tr>
<tr>
<td>250 µl of stock</td>
<td>250 µl</td>
<td>640 pg/ml</td>
</tr>
<tr>
<td>250 µl of 640 pg/ml</td>
<td>250 µl</td>
<td>320 pg/ml</td>
</tr>
<tr>
<td>250 µl of 320 pg/ml</td>
<td>250 µl</td>
<td>160 pg/ml</td>
</tr>
<tr>
<td>250 µl of 160 pg/ml</td>
<td>250 µl</td>
<td>80 pg/ml</td>
</tr>
<tr>
<td>250 µl of 80 pg/ml</td>
<td>250 µl</td>
<td>40 pg/ml</td>
</tr>
<tr>
<td>250 µl of 40 pg/ml</td>
<td>250 µl</td>
<td>20 pg/ml</td>
</tr>
</tbody>
</table>
Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Master Standard and/or diluted standard solutions.

Biotin Labelled Antibody Conc. (100x)
Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Dilution Buffer. Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Conc. (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)
Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures I-FABP in serum, plasma (EDTA, citrate, heparin) and urine.

Samples should be assayed immediately after collection, or should be stored frozen. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

An appropriate dilution should be assessed by the researcher prior to batch measurement.

Recommended starting dilution for serum and plasma is 5x.
Dilute samples 5x with Dilution Buffer just prior to the assay, e.g. 30 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 50 µl of sample + 200 µl of Dilution Buffer for duplicates. Mix well (not to foam). Vortex is recommended.

Recommended starting dilution for urine samples is 3x.

Stability and storage:
Samples should be stored at -20°C, or preferably at -70°C for long-term storage. Avoid repeated freeze/thaw cycles.
Do not store the diluted samples.

See Chapter 13 for effect of sample matrix (serum/plasma) on the concentration of human I-FABP.

*Note:* It is recommended to use a precise pipette and a careful technique to perform the dilution in order to get precise results.
11. ASSAY PROCEDURE

1. Pipet **100 µl** of diluted Standards, Dilution Buffer (=Blank) and diluted 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 **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Pipet **100 µl** of Biotin Labelled 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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
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 3-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution into each well. 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. Do not shake the plate during the incubation.
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. The absorbance should be read within **5 minutes following step 12**.

**Note 1:** If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine I-FABP concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

**Note 2:** Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat twice. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.
<table>
<thead>
<tr>
<th></th>
<th>strip 1+2</th>
<th>strip 3+4</th>
<th>strip 5+6</th>
<th>strip 7+8</th>
<th>strip 9+10</th>
<th>strip 11+12</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td><strong>Standard 1280</strong></td>
<td>Sample 1</td>
<td>Sample 9</td>
<td>Sample 17</td>
<td>Sample 25</td>
<td>Sample 33</td>
</tr>
<tr>
<td>B</td>
<td><strong>Standard 640</strong></td>
<td>Sample 2</td>
<td>Sample 10</td>
<td>Sample 18</td>
<td>Sample 26</td>
<td>Sample 34</td>
</tr>
<tr>
<td>C</td>
<td><strong>Standard 320</strong></td>
<td>Sample 3</td>
<td>Sample 11</td>
<td>Sample 19</td>
<td>Sample 27</td>
<td>Sample 35</td>
</tr>
<tr>
<td>D</td>
<td><strong>Standard 160</strong></td>
<td>Sample 4</td>
<td>Sample 12</td>
<td>Sample 20</td>
<td>Sample 28</td>
<td>Sample 36</td>
</tr>
<tr>
<td>E</td>
<td><strong>Standard 80</strong></td>
<td>Sample 5</td>
<td>Sample 13</td>
<td>Sample 21</td>
<td>Sample 29</td>
<td>Sample 37</td>
</tr>
<tr>
<td>F</td>
<td><strong>Standard 40</strong></td>
<td>Sample 6</td>
<td>Sample 14</td>
<td>Sample 22</td>
<td>Sample 30</td>
<td>Sample 38</td>
</tr>
<tr>
<td>G</td>
<td><strong>Standard 20</strong></td>
<td>Sample 7</td>
<td>Sample 15</td>
<td>Sample 23</td>
<td>Sample 31</td>
<td>Sample 39</td>
</tr>
<tr>
<td>H</td>
<td><strong>Blank</strong></td>
<td>Sample 8</td>
<td>Sample 16</td>
<td>Sample 24</td>
<td>Sample 32</td>
<td>Sample 40</td>
</tr>
</tbody>
</table>

Figure 1: Example of a work sheet.
12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of I-FABP (pg/ml) in samples.

Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of standards).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 100 pg/ml (from standard curve) x 5 (dilution factor) = 500 pg/ml

![Human I-FABP ELISA Standard Curve](image)

*Figure 2: Typical Standard Curve for Human I-FABP ELISA.*
13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Intestinal FABP (FABP2) ELISA are presented in this chapter.

- **Sensitivity**
  Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real I-FABP values in wells and is 3.5 pg/ml.
  *Dilution Buffer is pipetted into blank wells.

- **Limit of assay**
  Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

- **Specificity**
  The antibodies used in this ELISA are specific for human Intestinal FABP (FABP2) with no detectable crossreactivities to human Liver FABP (FABP1), Heart FABP (FABP3), Adipocyte FABP (FABP4), Epidermal FABP (FABP5), Ileal FABP (FABP6), Brain FABP (FABP7), Myelin FABP (FABP8), Testis FABP (FABP9) and FABP12.

Presented results are multiplied by respective dilution factor

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>697</td>
<td>27</td>
<td>3.8</td>
</tr>
<tr>
<td>Serum 2</td>
<td>914</td>
<td>39</td>
<td>4.3</td>
</tr>
</tbody>
</table>

Inter-assay (Run-to-Run) (n=6)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean (pg/ml)</th>
<th>SD (pg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td>1083</td>
<td>35</td>
<td>3.2</td>
</tr>
<tr>
<td>Serum 2</td>
<td>2289</td>
<td>118</td>
<td>5.1</td>
</tr>
</tbody>
</table>
- **Spiking Recovery**
  Samples were spiked with different amounts of human I-FABP and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>377</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>615</td>
<td>617</td>
<td>99.7</td>
</tr>
<tr>
<td></td>
<td>845</td>
<td>857</td>
<td>98.7</td>
</tr>
<tr>
<td></td>
<td>1225</td>
<td>1337</td>
<td>91.6</td>
</tr>
<tr>
<td>Serum 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>548</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>783</td>
<td>788</td>
<td>99.5</td>
</tr>
<tr>
<td></td>
<td>966</td>
<td>1028</td>
<td>94.0</td>
</tr>
<tr>
<td></td>
<td>1404</td>
<td>1508</td>
<td>93.1</td>
</tr>
</tbody>
</table>

- **Linearity**
  Samples were serially diluted with Dilution Buffer and assayed.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Observed (pg/ml)</th>
<th>Expected (pg/ml)</th>
<th>Recovery O/E (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2218</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>1188</td>
<td>1109</td>
<td>107.2</td>
</tr>
<tr>
<td></td>
<td>4x</td>
<td>591</td>
<td>554</td>
<td>106.6</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>284</td>
<td>277</td>
<td>102.3</td>
</tr>
<tr>
<td>Serum 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>7269</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2x</td>
<td>3613</td>
<td>3635</td>
<td>99.4</td>
</tr>
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<td></td>
<td>4x</td>
<td>1790</td>
<td>1817</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>8x</td>
<td>923</td>
<td>909</td>
<td>101.6</td>
</tr>
</tbody>
</table>
Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

<table>
<thead>
<tr>
<th>Volunteer No.</th>
<th>Serum (pg/ml)</th>
<th>Plasma (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>EDTA</td>
</tr>
<tr>
<td>1</td>
<td>645</td>
<td>598</td>
</tr>
<tr>
<td>2</td>
<td>609</td>
<td>554</td>
</tr>
<tr>
<td>3</td>
<td>2552</td>
<td>2084</td>
</tr>
<tr>
<td>4</td>
<td>389</td>
<td>348</td>
</tr>
<tr>
<td>5</td>
<td>1221</td>
<td>1170</td>
</tr>
<tr>
<td>6</td>
<td>146</td>
<td>132</td>
</tr>
<tr>
<td>7</td>
<td>1130</td>
<td>1031</td>
</tr>
<tr>
<td>8</td>
<td>3220</td>
<td>3149</td>
</tr>
<tr>
<td>9</td>
<td>299</td>
<td>214</td>
</tr>
<tr>
<td>10</td>
<td>328</td>
<td>286</td>
</tr>
</tbody>
</table>

Mean (pg/ml): 1054, 957, 817, 1014

Mean Plasma/Serum (%): 90.8, 77.5, 96.2

Coefficient of determination $R^2$: 0.99, 0.98, 0.96

Figure 3: I-FABP levels measured using Human Intestinal FABP (FABP2) ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.
14. DEFINITION OF THE STANDARD

Recombinant human I-FABP is used as the standard. The recombinant human I-FABP produced in E. coli is a 16.32 kDa protein containing 131 amino acid residues of human I-FABP and 10 extra AA.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 155 unselected donors (89 men + 66 women) 21-65 years old were assayed with the BioVendor Human Intestinal FABP (FABP2) ELISA in our laboratory.

- Age dependent distribution of I-FABP

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age (years)</th>
<th>n</th>
<th>I-FABP (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Men</td>
<td>21-29</td>
<td>18</td>
<td>1037</td>
</tr>
<tr>
<td></td>
<td>30-39</td>
<td>26</td>
<td>1343</td>
</tr>
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</table>
Figure 4: Human I-FABP concentration plotted against donor age and sex.

- **Reference range**
  The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological references ranges for I-FABP levels with the assay.
16. METHOD COMPARISON

The BioVendor Human Intestinal FABP (FABP2) ELISA was compared to another commercial immunoassay by measuring 48 serum samples. The following correlation graph was obtained:
17. TROUBLESHOOTING AND FAQS

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
- Improper wavelength when reading absorbance

High signal and background in all wells
Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)
Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards and samples
REFERENCES


For more references on this product see our WebPages at www.biovendor.com
## 19. EXPLANATION OF SYMBOLS

<table>
<thead>
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<td>Content</td>
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<tr>
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<td>Lot number</td>
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<td>Attention, see instructions for use</td>
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<td>Potential biological hazard</td>
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<tr>
<td>🕒</td>
<td>Expiry date</td>
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<td>Storage conditions</td>
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<tr>
<td>⛰️ 2 °C</td>
<td>Name and registered office of the manufacturer</td>
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Antibody Coated Microtiter Plate

Reconstitute Master Standard, prepare set of standards

Add standards and samples
100 µl

Dilute samples

Incubate at RT for 1 hour / 300 rpm

Prepare Wash Solution

Wash 3x

Prepare Biotin Labeled Antibody solution

Add Biotin Labelled Antibody solution
100 µl

Incubate at RT for 1 hour / 300 rpm

Add Streptavidin HRP Conjugate
100 µl

Incubate at RT for 30 min / 300 rpm

Add Substrate Solution
100 µl

Incubate at RT for 10 min

Add Stop Solution
100 µl

Read absorbance and calculate results

Assay Procedure Summary