

ENG

Instructions for use:
**HUMAN INTESTINAL FABP
(FABP2) ELISA**

Catalogue number:
RD191246200R

For research use only!

 **BioVendor**
R&D®

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HISTORY OF CHANGES

Previous version	Current Version
ENG.003.A	ENG.004.A
"History of changes" added.	
Chapter 9: A sentence „Centrifuge liquid containing microtube vials before opening“ added.	

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

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	2x 20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

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 μ l with disposable tips
- Multichannel pipette to deliver 100 μ l with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtiter 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.

Centrifuge liquid containing microtube vials before opening,

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:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	1280 pg/ml
250 µl of stock	250 µl	640 pg/ml
250 µl of 640 pg/ml	250 µl	320 pg/ml
250 µl of 320 pg/ml	250 µl	160 pg/ml
250 µl of 160 pg/ml	250 µl	80 pg/ml
250 µl of 80 pg/ml	250 µl	40 pg/ml
250 µl of 40 pg/ml	250 µl	20 pg/ml

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.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Standard 1280	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	Standard 640	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	Standard 320	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	Standard 160	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	Standard 80	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	Standard 40	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	Standard 20	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
H	Blank	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40

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.

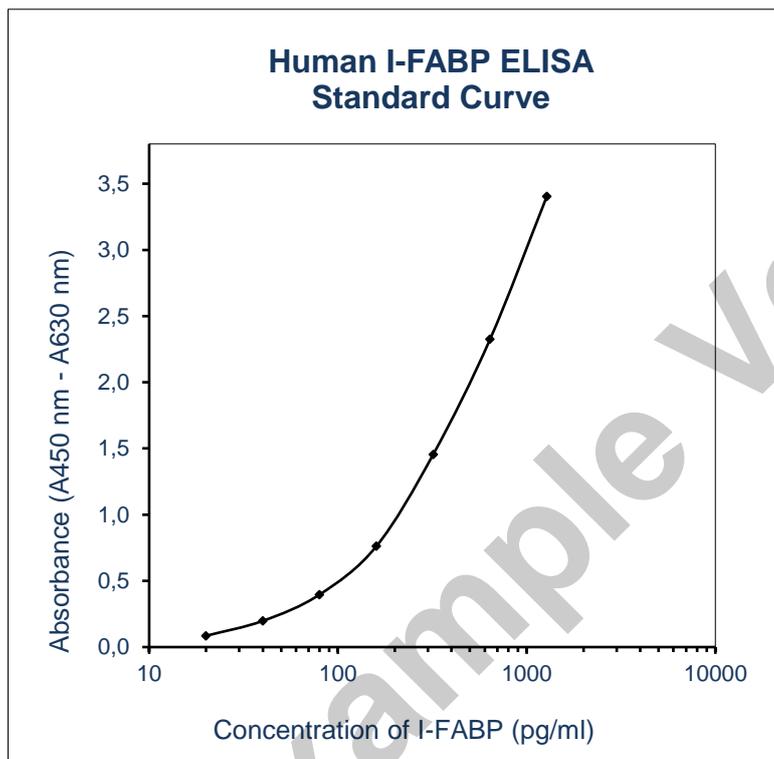


Figure 2: Typical Standard Curve for Human I-FABP ELISA.

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: $A_{\text{blank}} + 3 \times \text{SD}_{\text{blank}}$) 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)

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Serum 1	697	27	3.8
Serum 2	914	39	4.3

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

Sample	Mean (pg/ml)	SD (pg/ml)	CV (%)
Serum 1	1083	35	3.2
Serum 2	2289	118	5.1

Spiking Recovery

Samples were spiked with different amounts of human I-FABP and assayed.

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

Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (pg/ml)	Expected (pg/ml)	Recovery O/E (%)
Serum 1	-	2218	-	-
	2x	1188	1109	107.2
	4x	591	554	106.6
	8x	284	277	102.3
Serum 2	-	7269	-	-
	2x	3613	3635	99.4
	4x	1790	1817	98.5
	8x	923	909	101.6

Effect of sample matrix

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

Volunteer No.	Serum (pg/ml)	Plasma (pg/ml)		
		EDTA	Citrate	Heparin
1	645	598	441	592
2	609	554	469	575
3	2552	2084	1793	2001
4	389	348	319	345
5	1221	1170	1022	1439
6	146	132	109	160
7	1130	1031	823	967
8	3220	3149	2779	3475
9	299	214	186	255
10	328	286	228	333
Mean (pg/ml)	1054	957	817	1014
Mean Plasma/Serum (%)	-	90.8	77.5	96.2
Coefficient of determination R²	-	0.99	0.98	0.96

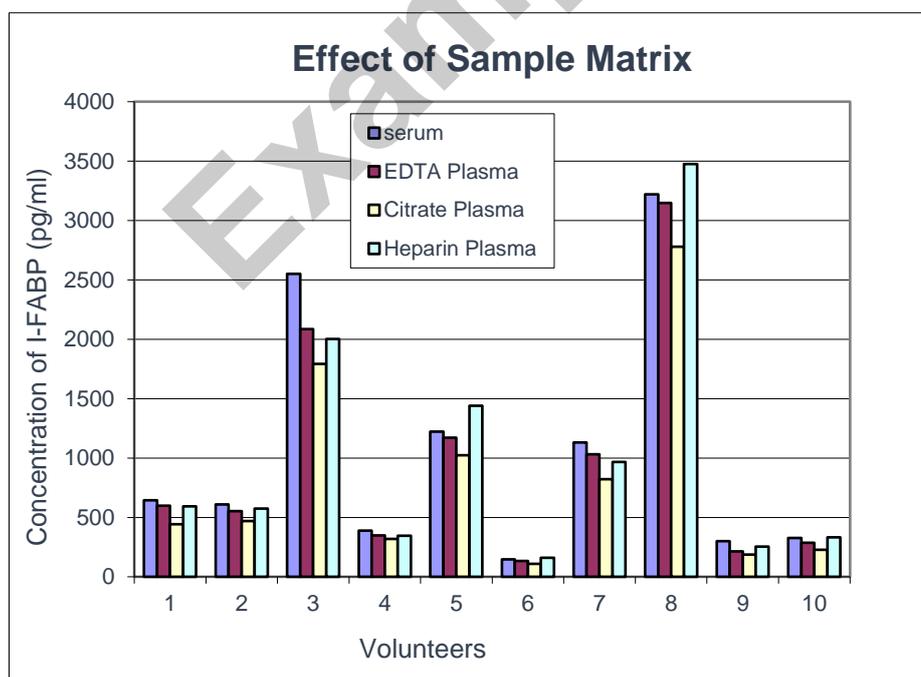


Figure 3: I-FABP levels measured using Human Intestinal FABP (FABP2) ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

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

Sex	Age (years)	n	I-FABP (pg/ml)				
			Mean	Median	SD	Min	Max
Men	21-29	18	1037	896	548	291	2177
	30-39	26	1343	879	1566	118	8407
	40-49	31	1122	901	746	375	3920
	50-65	14	1160	957	743	372	3282
Women	22-29	12	926	860	511	340	2025
	30-39	26	795	756	484	94	2146
	40-49	20	967	766	503	268	2050
	50-61	8	1515	1246	529	461	3387

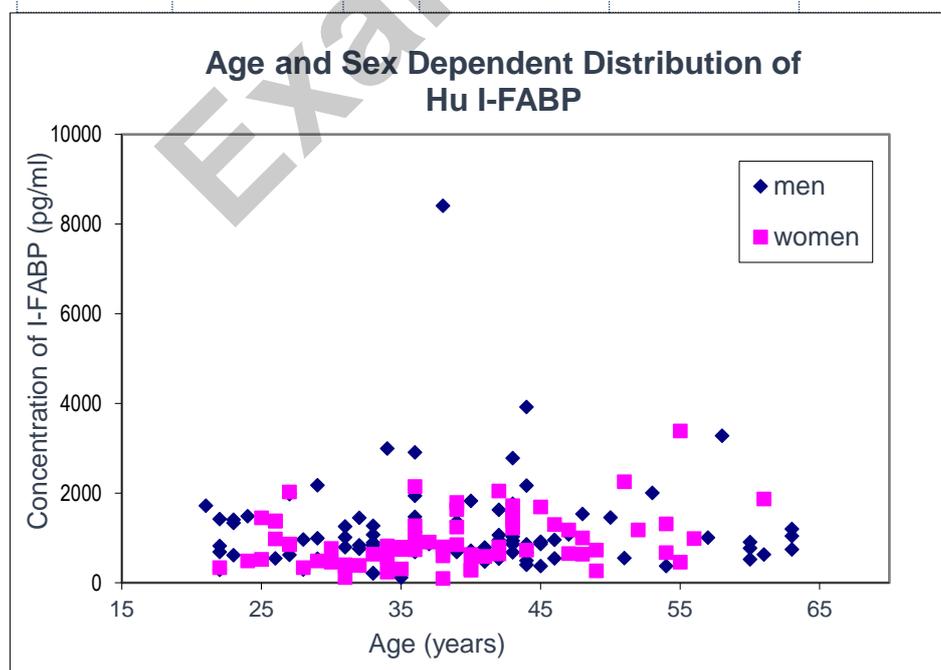


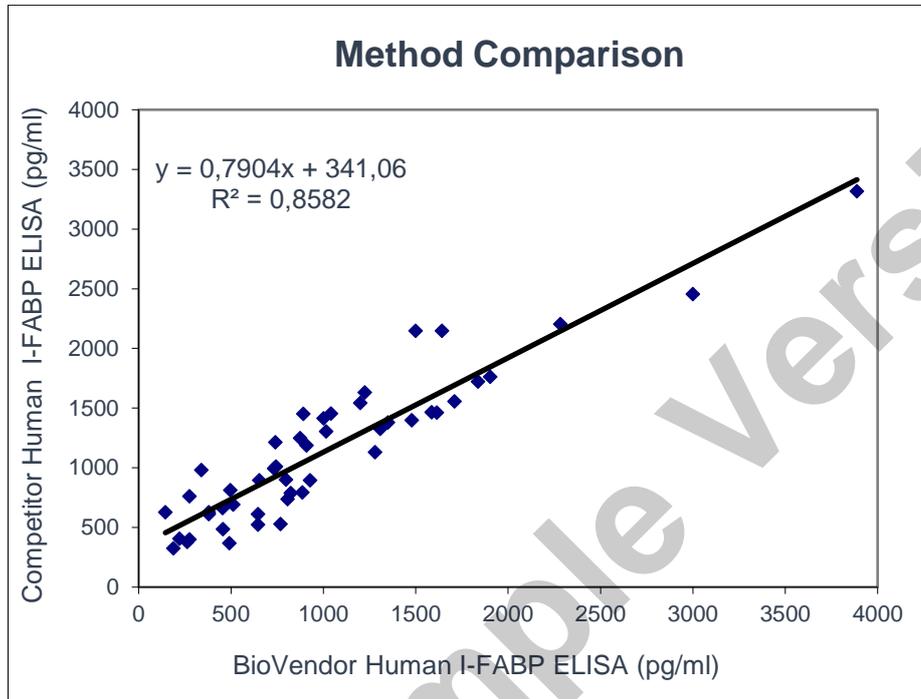
Figure 4: Human I-FABP concentration plotted against donor age and sex.

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
-

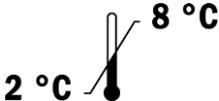
Example Version

18. REFERENCES

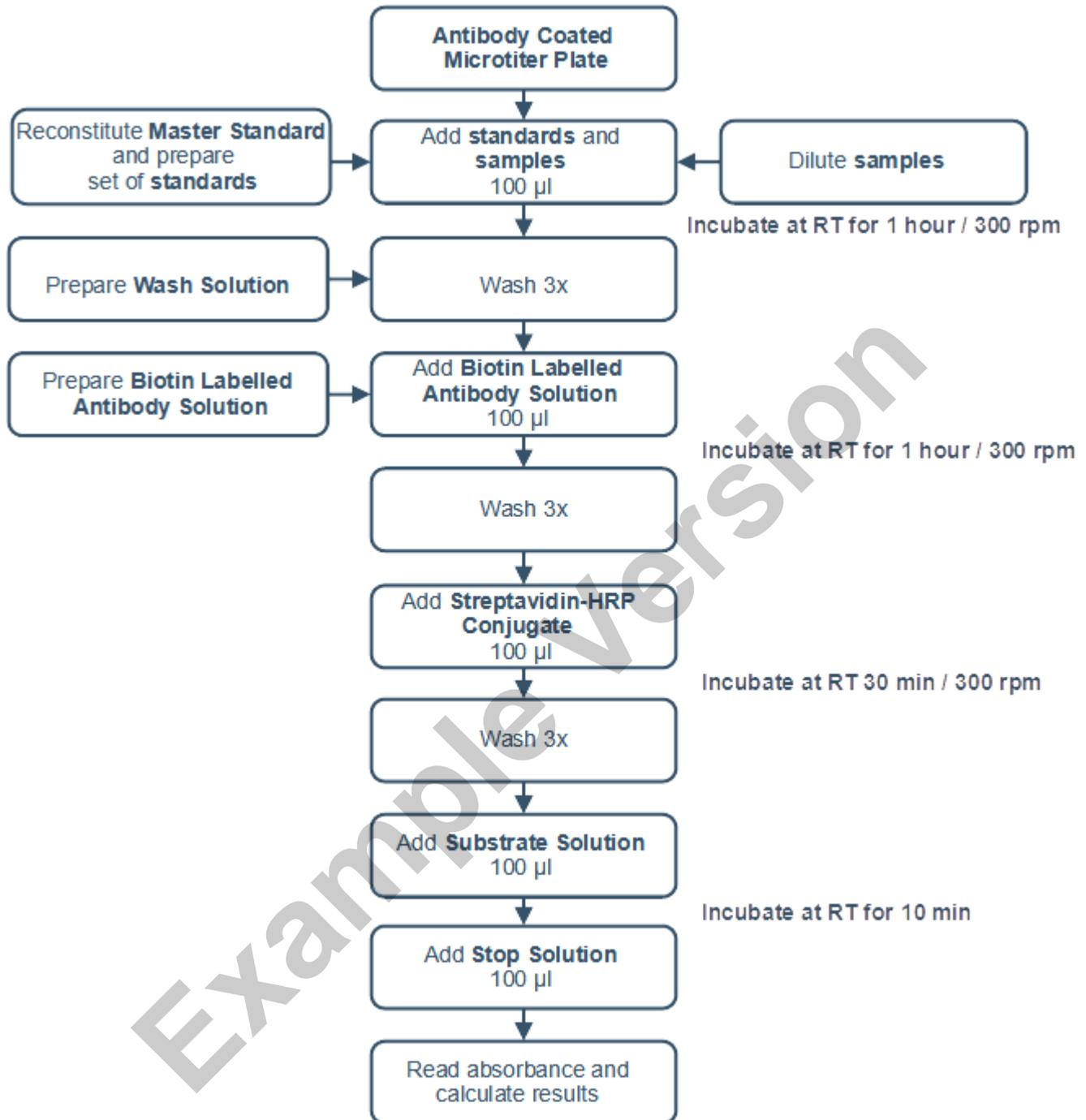
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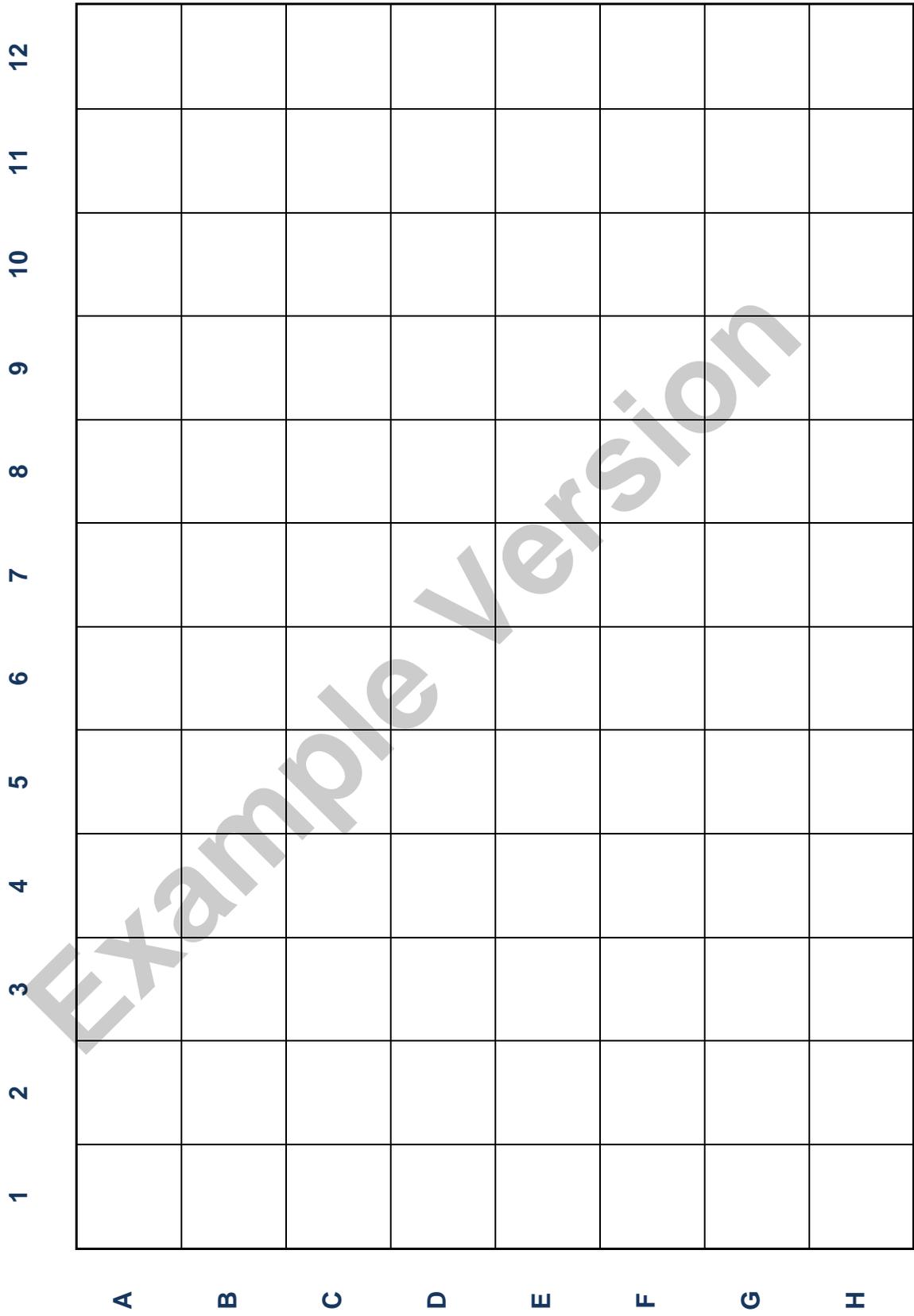
For more references on this product see our web pages at www.biovendor.com.

19. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

20. ASSAY PROCEDURE - SUMMARY







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