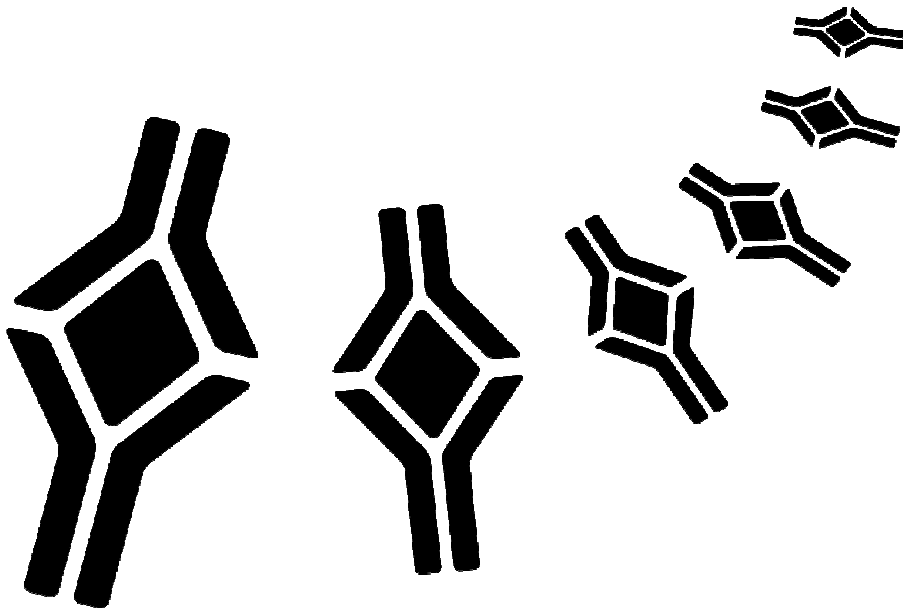


BioVendor

Research
and Diagnostic Products



HUMAN FETUIN-B ELISA

Product Data Sheet

Cat. No.: RD191172200R

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 RD191172200R Human Fetuin-B ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human fetuin-B.

»» Features

- **It is intended for research use only**
- The total assay time is less than 4 hours
- The kit measures fetuin-B in human serum and plasma (EDTA, citrate, heparin)
- 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

The protein fetuin-B encoded by the FETUB gene is a member of the fetuin family, part of the cystatin superfamily of cysteine protease inhibitors.

By searching DNA sequence databases, expressed sequence tags encoding human and mouse fetuin-B were identified. The 382 amino acid human fetuin-B protein shares 24% sequence similarity with fetuin-A, the prototypic member of the fetuin protein family. The human, mouse and rat fetuin-B proteins share 61% amino acid identity [1, 2].

The expression of fetuin-B is regulated by farnesoid X receptor (FXR), a nuclear receptor that acts as a key factor in the regulation of bile acid, lipid and carbohydrate metabolism [3].

Functional analysis revealed that fetuin-B, similarly to fetuin-A, is an inhibitor of basic calcium phosphate precipitation, however its activity is lower compared to fetuin-A [4].

Comparison of the expression levels of fetuin-B and fetuin-A at the RNA level revealed that both fetuin genes are most highly expressed in liver tissue. Like fetuin-A, fetuin-B mRNA was found to be highly expressed in tongue and placenta tissues. Fetuin-B is also expressed at the protein level in sera and several organs of mouse, rat and human. Unlike fetuin-A the amount of fetuin-B protein in human serum varied with gender and was higher in females than in males [4].

Results obtained from proteomic studies on rats provided a possible relationship between reduced plasma protein levels of fetuin-B and Zinc- α 2-glycoprotein (ZAG) and higher risk of diet induced obesity through impaired fatty acid metabolism in hepatocytes [5].

Plasma fetuin-B along with other proteins was differentially regulated between healthy control and streptozotocin-induced male and female diabetic rats. The proteomic data on gender-dimorphic regulation of plasma proteins can provide valuable information that may be used for evidence-based gender-specific clinical treatment of diabetes [6].

In a different study, elevated fetuin-B levels were observed in CSF of rats with experimentally induced autoimmune encephalomyelitis (EAE). The EAE model resembles certain aspects of multiple sclerosis, with common features such as motor dysfunction, axonal degradation, and infiltration of T-cells [7].

The fetuins have also been shown to be subject to inflammation associated changes in hepatic mRNA expression. Fetuin-A and -B are negative acute-phase proteins, meaning that their hepatic expression is down-regulated in response to an inflammatory stimulus [8, 9]. In an *in vitro* model, fetuin-B was found to be significantly down-regulated in hepatocytes under a high dose of acoustic nanobubbles. In this context, immunohistochemistry detected liver fibrosis and inflammation with nanobubble treatment [10].

Genetic association studies have reported tumor suppressor activity and that overexpression of fetuin-B in skin squamous carcinoma cells suppresses tumor growth in nude mice [11].

Targeted gene deletion of fetuin-B in mice causes premature ZP (zona pellucida) hardening and, consequently, female infertility. Transplanting fetuin-B-deficient ovaries into wild-type recipients restores fertility to the ovaries, indicating that plasma fetuin-B is necessary and sufficient for fertilization [12].

A recent study which analysed differentially expressed proteins in serum between, before and after transection of the anterior cruciate ligament found increased levels of fetuin-B in dogs with osteoarthritis compared to healthy dogs [13]. In dogs, Fetuin B was also identified as one of seven proteins elevated in urine of animals bitten by the European adder [14].

Areas of investigation:

Energy metabolism and body weight regulation

Inflammatory diseases

Oncology

Bone metabolism

4. TEST PRINCIPLE

In the BioVendor Human Fetuin-B ELISA, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-human fetuin-B antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-human fetuin-B antibody is added and incubated with the captured fetuin-B for 60 minutes. 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 fetuin-B. A standard curve is constructed by plotting absorbance values against fetuin-B 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 mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

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

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision 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 microtiterate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of 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 stored at 2–8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Biotin-Ab Diluent

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:

Dilution Buffer Conc. (10x)

Dilute Dilution Buffer Concentrate (10x) ten-fold in 180 ml distilled water to prepare a 1x working solution, e.g. 20 ml of Dilution Buffer Concentrate (10x) + 180 ml of distilled water for use of all 96-wells.

It is recommended to dilute only such a volume of Dilution Buffer Concentrate (10x) to be used up in the one run of the test.

Stability and storage:

The diluted Dilution Buffer is stable 1 week when stored at 2–8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2–8°C.

Human Fetuin-B 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 30 minutes with occasional gentle shaking (not to foam). The resulting concentration of the human fetuin-B in the stock solution is **4 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

<i>Volume of Standard</i>	<i>Dilution Buffer</i>	<i>Concentration</i>
Stock	–	4 ng/ml
250 µl of stock	250 µl	2 ng/ml
250 µl of 2 ng/ml	250 µl	1 ng/ml
250 µl of 1 ng/ml	250 µl	0.5 ng/ml
250 µl of 0.5 ng/ml	250 µl	0.25 ng/ml
250 µl of 0.25 ng/ml	250 µl	0.125 ng/ml

Prepared standards are ready to use, do not dilute them.

Stability and storage:

Do not store the reconstituted Master Standard and diluted standard solutions.

Biotin Labelled Antibody

Refer to the Certificate of Analysis for current volume of Biotin-Ab Diluent needed for reconstitution of Biotin Labelled Antibody!!!

Reconstitute the lyophilized Biotin Labelled Antibody with Biotin-Ab Diluent just prior to the assay. Let it dissolve at least 30 minutes with occasional gentle shaking (not to foam).

Dilute Biotin Labelled Antibody Concentrate 100x with Biotin-Ab Diluent (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Biotin-Ab Diluent for 8 wells).

Stability and storage:

Do not store 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 human fetuin-B in serum and plasma (EDTA, citrate, heparin)

Samples can be assayed immediately after collection, or after long-term storage at -20°C or -70°C. 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 in advance to batch measurement. Recommended starting dilution is 2000x.

Dilute samples (serum, plasma) 2000x with the Dilution Buffer just prior to the assay in two steps as follows:

Dilution A (50x):

Add 10 µl of sample into 490 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Dilution B (40x):

Add 10 µl of Dilution A into 390 µl of Dilution Buffer for duplicates to prepare final dilution (2000x). **Mix well** (not to foam). Vortex is recommended.

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 fetuin-B.

Note: It is recommended to use a precision 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. Add **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. Add **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 **15 minutes** at room temperature. The incubation time may be extended [up to 25 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 absorbance 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 fetuin-B 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 4	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
B	Standard 2	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
C	Standard 1	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
D	Standard 0.5	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
E	Standard 0.25	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
F	Standard 0.125	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39
G	Blank	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40
H	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	Sample 41

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 fetuin-B (ng/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 they have been diluted prior to the assay, e.g. 1,5 ng/ml (from standard curve) x 2000 (dilution factor) = 3000 ng/ml.

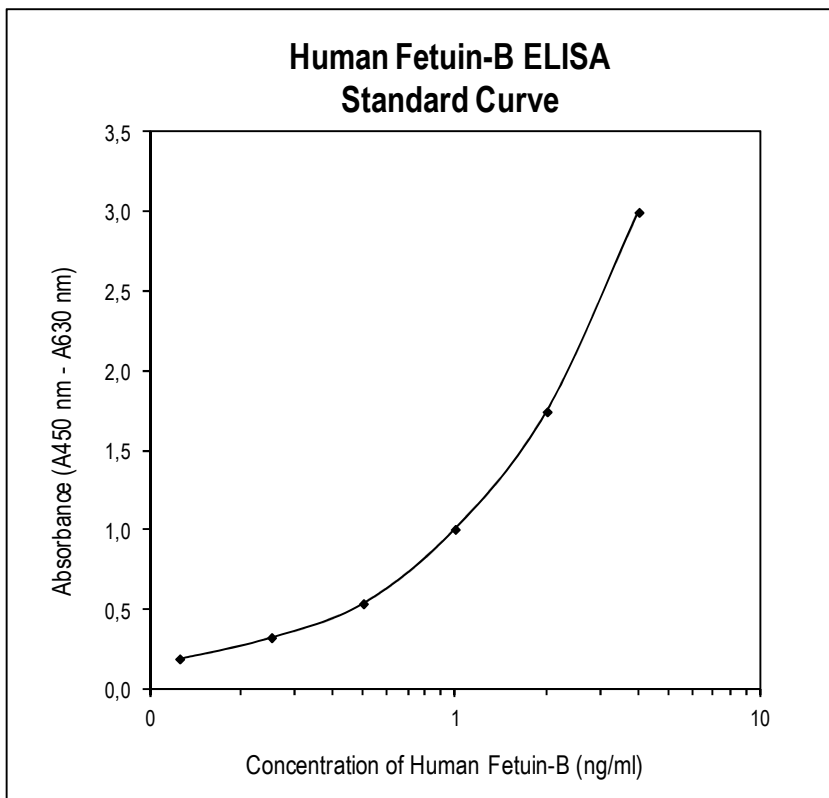


Figure 2: Typical standard curve for Human Fetuin-B ELISA.

13. PERFORMANCE CHARACTERISTICS

➤➤ Typical analytical data of BioVendor Human Fetuin-B 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 human fetuin-B values in wells and is 0.019 ng/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 fetuin-B with no detectable crossreactivity to human fetuin-A at 100 µg/ml and human cystatin C at 10 µg/ml.

➤➤ Presented results are multiplied by respective dilution factor

- **Precision**

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
Serum 1	2129	111	5.19
Serum 2	3031	66	2.18

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

<i>Sample</i>	<i>Mean (ng/ml)</i>	<i>SD (ng/ml)</i>	<i>CV (%)</i>
Serum 1	1185	52	4.40
Serum 2	5535	337	6.09

- **Spiking Recovery**

Serum samples were spiked with different amounts of human fetuin-B and assayed.

<i>Sample</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
Serum 1	1080	-	-
	1612	1580	102.0
	2142	2080	103.0
	2976	3080	96.6
Serum 2	748	-	-
	1157	1248	92.7
	1706	1748	97.6
	2510	2748	91.3

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

<i>Sample</i>	<i>Dilution</i>	<i>Observed (ng/ml)</i>	<i>Expected (ng/ml)</i>	<i>Recovery O/E (%)</i>
Serum 1	-	2826	-	-
	2x	1516	1413	107.3
	4x	763	706	108.0
	8x	329	353	93.2
Serum 2	-	3753	-	-
	2x	1922	1876	102.4
	4x	1010	938	107.6
	8x	509	469	108.5

- **Effect of sample matrix**

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

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	1621	2059	1658	1947
2	2875	3230	2755	2942
3	3089	3648	2756	3198
4	4664	4793	4456	4864
5	3966	3572	3347	3175
6	1642	1817	1270	1563
7	2131	1931	1490	1783
8	1430	1373	1413	1542
9	1711	1982	1671	1957
10	1569	1833	1457	1835
Mean (ng/ml)	2470	2624	2227	2481
Mean Plasma/Serum (%)		106.2	90.2	100.4
Coefficient of determination R²		0.93	0.91	0.96

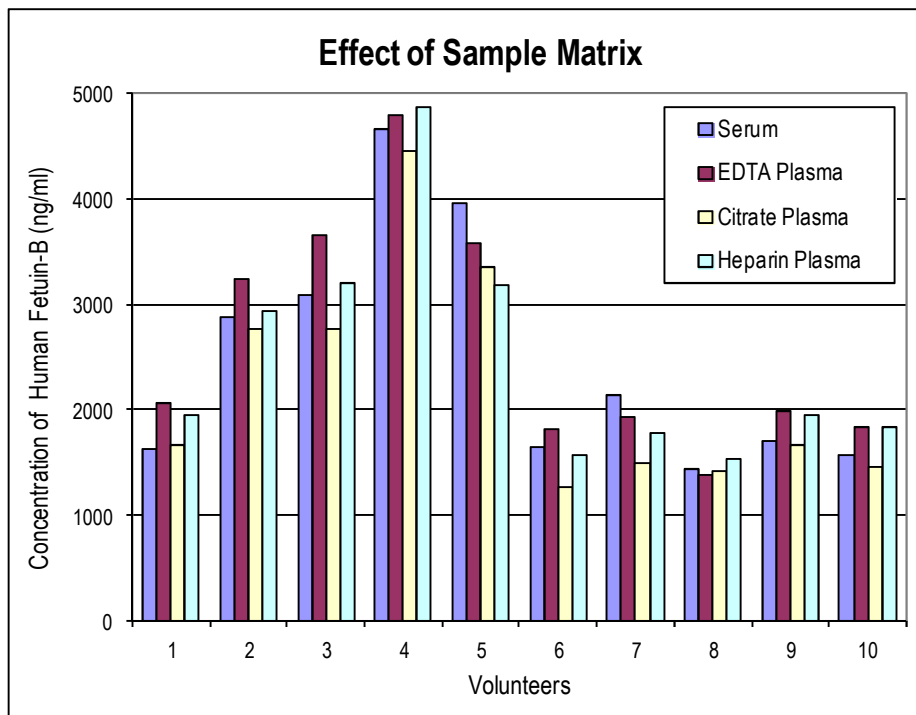


Figure 3: Fetuin-B levels measured using Human Fetuin-B ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

In this assay the recombinant protein (HEK293) is used as the standard. The recombinant fetuin-B is a 41.8 kDa protein consisting of 367 amino acid residues of the human fetuin-B and 10 extra aminoacids.

15. PRELIMINARY POPULATION 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 Fetuin-B ELISA in our laboratory.

Sex	Age (years)	n	Mean	Median	SD	Min	Max
			Fetuin-B (ng/ml)				
Men	21-29	17	2000	1951	612	1052	3177
	30-39	25	2216	2231	529	1095	3341
	40-49	31	2010	1959	604	682	3408
	50-65	16	1829	1862	375	787	2446
Women	22-29	12	3404	3434	1264	1398	5052
	30-39	26	2696	2207	1076	1434	4975
	40-49	20	2580	2246	1189	1064	6175
	50-61	8	2199	2263	515	1303	3153

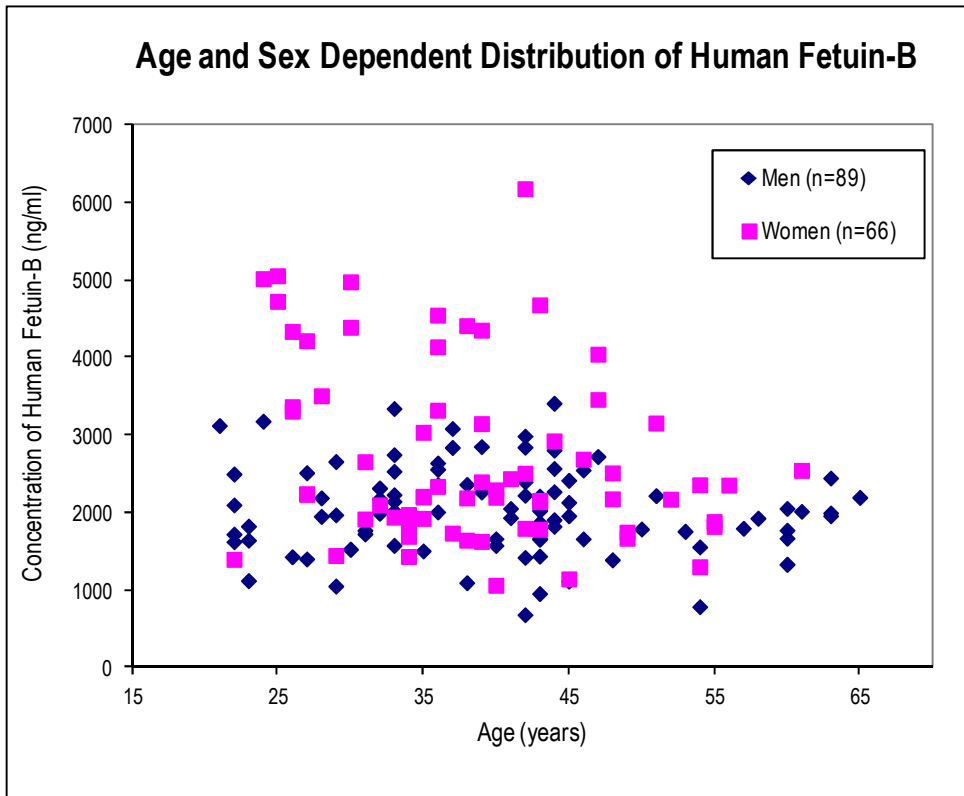


Figure 4: Human fetuin-B concentration plotted against donor age and sex.

- **Reference range**

It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for fetuin-B levels with the assay.

16. 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 or samples









17. REFERENCES

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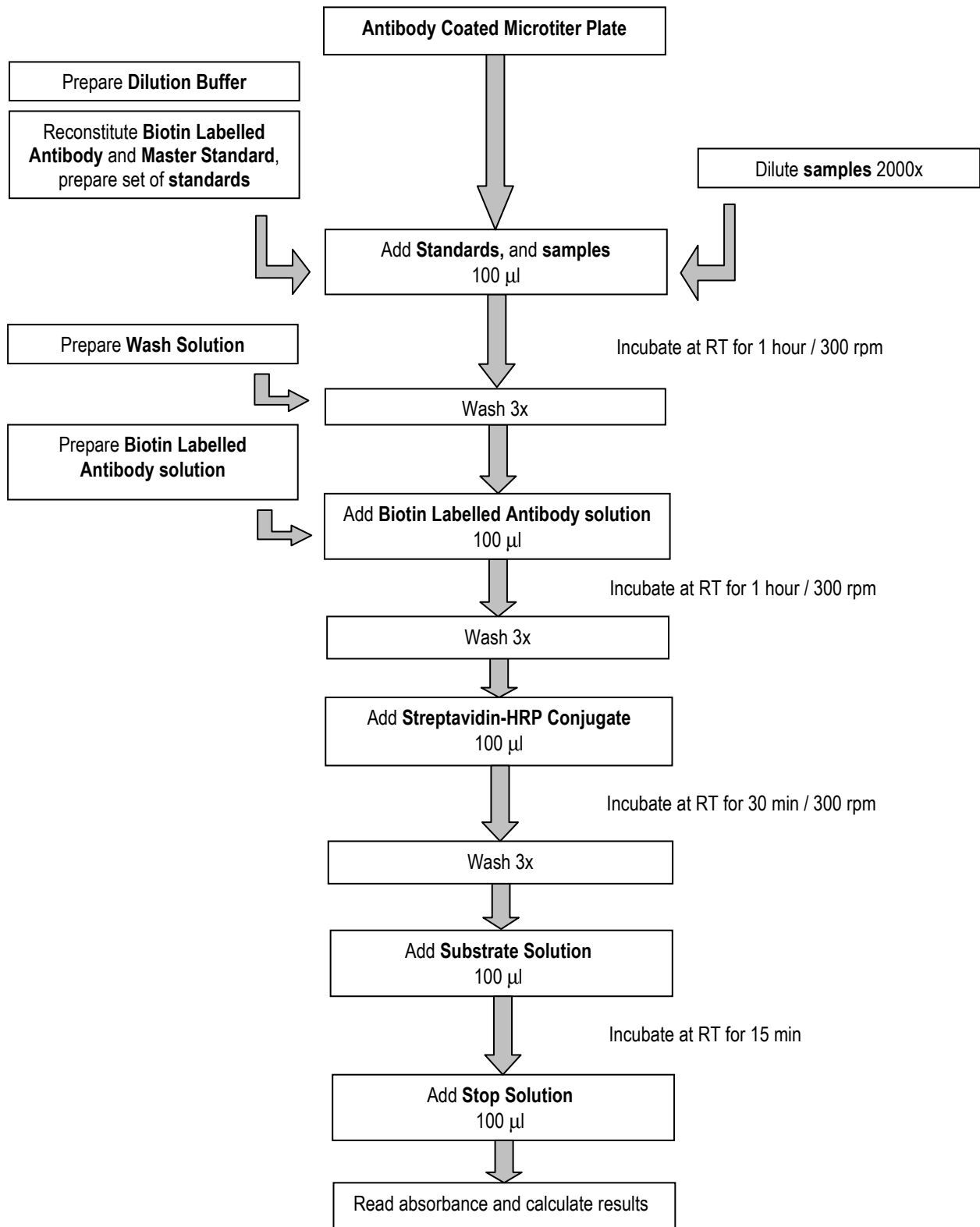
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➤➤ For more references on this product see our WebPages at www.biovendor.com

18. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials

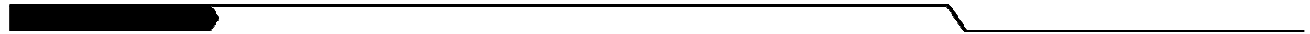
Assay Procedure Summary



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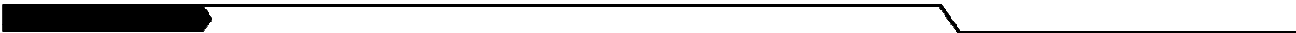
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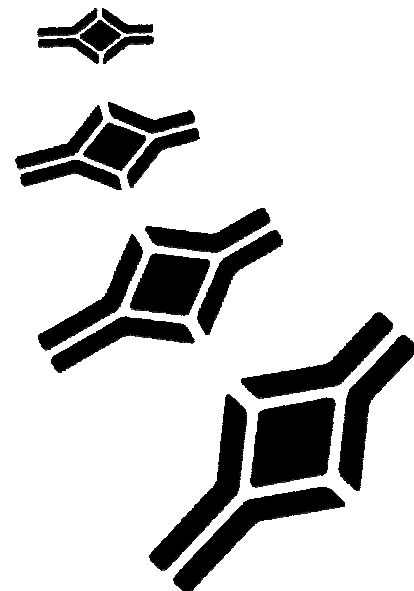


NOTES



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