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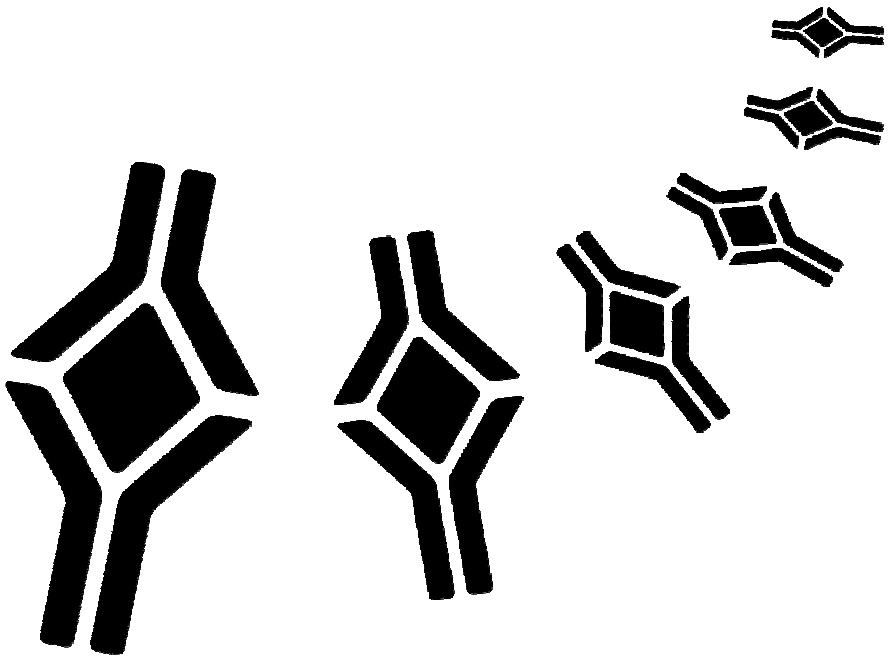

HUMAN FETUIN-A ELISA

Product Data Sheet

Cat. No.: RD191037100

European Union: IVD CE

Rest of the world:
For research use only!

VERSION 41 160311 07

CONTENTS

» **This kit is manufactured by:**
BioVendor – Laboratorni medicina a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

1. INTENDED USE

The RD191037100 Human Fetuin-A ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human fetuin-A.

Features

- **European Union: for in vitro diagnostic use**
  Rest of the world: for research use only!
- The total assay time is less than 3 hours
- The kit measures fetuin-A in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based. No animal sera are used
- Standard is human plasma 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

Fetuin-A (AHSG), a 59 kDa glycoprotein, consists of two cystatin-like domains and a smaller unrelated domain. Its homologues have been identified in several species including rat (pp63), mouse, guinea pig, rabbit, sheep, cattle, swine and human. AHSG human gene is located on chromosome 3q27.
Liver synthesized fetuin-A is secreted into the blood stream and it is deposited as a noncollagenous protein in mineralized bones and teeth.
Fetuin-A occurs in high serum concentration during fetal life, whereas its level declines following infection, inflammation (by 20-30% during acute phase) and malignancy. Fetuin-A may influence the resolution of inflammation by modulating the phagocytosis of apoptotic cells by macrophages.
Fetuin-A acts as an important circulating inhibitor of ectopic calcification that is a frequent complication of many degenerative diseases.
The low fetuin-A level may be associated with higher cardiovascular mortality in chronic renal failure, liver cancer and liver cirrhosis patients on long-term dialysis.
Human fetuin-A represents a natural inhibitor of tyrosine kinase activity of the insulin receptor.
Fetuin-A may play a significant role in regulating postprandial glucose disposal, insulin sensitivity, weight gain, and fat accumulation and may be a novel therapeutic target in the treatment of type 2 diabetes, obesity, and other insulin-resistant conditions.
The serum and bone-resident fetuin-A binds to transforming growth factor-β and blocks TGF-β binding to cell surface receptors.
Thus, fetuin-A is involved at least in:

- inhibition of unwanted (vascular) calcification
- inhibition of insulin receptor tyrosine kinase activity
- regulation of osteogenesis

4. TEST PRINCIPLE

In the BioVendor Human Fetuin-A ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human fetuin-A antibody. After 60 minutes incubation and washing, polyclonal anti-human fetuin-A antibody, conjugated with horseradish peroxidase (HRP) is added to the wells and incubated for 60 minutes with captured fetuin-A. Following another washing step, the remaining HRP 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-A. 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 contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. These materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- 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

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Conjugate Solution	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
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 10-1000 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 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 550-650nm)
- 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 month stored at 2-8°C and protected from the moisture.

Conjugate Solution
Substrate Solution
Stop Solution
Stability and storage:
Opened reagents are stable 3 month when stored at 2-8°C.

- **Assay reagents supplied concentrated or lyophilized:**

Dilution Buffer Conc. (10x)
Dilute Dilution Buffer Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 20 ml of Dilution Buffer Concentrate (10x) + 180 ml of distilled water for use of all 96-wells.
Stability and storage:
The diluted Dilution Buffer is stable 1 month when stored at 2-8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2-8°C.

Human Fetuin-A 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 the fetuin-A in the stock solution is **100 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	100 ng/ml
200 µl of stock	300 µl	40 ng/ml
250 µl of 40 ng/ml	250 µl	20 ng/ml
250 µl of 20 ng/ml	250 µl	10 ng/ml
250 µl of 10 ng/ml	250 µl	5 ng/ml
200 µl of 5 ng/ml	300 µl	2 ng/ml

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

Stability and storage:

Reconstituted Master Standard (100 ng/ml) must be used immediately or aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!

Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Stability and storage:

Do not store the reconstituted Quality Controls.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) 10-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 fetuin-A in serum and plasma (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored at -20°C. Mix thoroughly thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic samples.

Dilute samples **10 000x** with Dilution Buffer just prior to the assay in two steps as follows:
Dilution A (100x):
Add 10 µl of sample into 990 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.
Dilution B (100x):
Add 10 µl of Dilution A into 990 µl of Dilution Buffer to prepare final dilution (10 000x) and **mix well** (not to foam). Vortex is recommended.
Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of fetuin-A.

Ask for protocol at info@biovendor.com if assaying other samples.

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, Quality Controls, Dilution Buffer (=Blank) and 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 Conjugate 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 Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. 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.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. 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 9.**

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 fetuin-A 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 100**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 40**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 20**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 10**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 2**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

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-A (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 samples s have been diluted prior to the assay, e.g. 21.5 ng/ml (from standard curve) x 10 000 (dilution factor) = 215.0 µg/ml or 0.215 g/l.

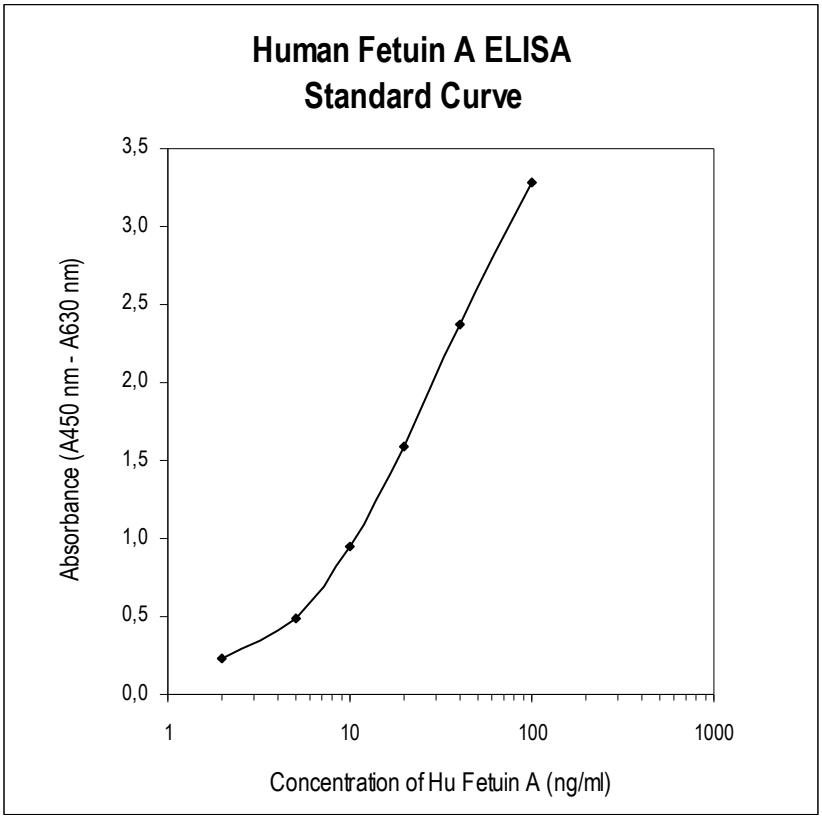

Figure 2: Typical Standard Curve for Human Fetuin-A ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Fetuin-A 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 fetuin-A values in wells and is 0.35 ng/ml .
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

Results exceeding fetuin-A level of 100 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the fetuin-A concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human fetuin-A.

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

» **Presented results are multiplied by respective dilution factor**

- **Precision**

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

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	104.5	0.41	3.9
2	444.8	2.89	6.5

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

Sample	*Mean (µg/ml)*	*SD (µg/ml)*	*CV (%)*
1	128.3	0.65	5.1
2	271.8	0.69	2.6

- **Spiking Recovery**

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

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	62.5	-	-
	110.5	112.5	98.2
	175.3	162.5	107.9
	251.2	262.5	95.7
2	102.0	-	-
	144.5	152.0	95.1
	194.4	202.0	96.2
	269.5	302.0	89.2

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	-	248.9	-	-
	2x	121.7	124.5	97.8
	4x	57.6	62.2	92.6
	8x	27.6	31.1	88.7
2	-	510.9	-	-
	2x	267.6	255.5	104.8
	4x	143.5	127.7	112.4
	8x	68.6	63.9	107.4

- **Effect of sample matrix**

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

Volunteer No.	*Serum (µg/ml)*	*Plasma (µg/ml)*		
		EDTA	*Citrate*	*Heparin*
1	180.5	184.0	148.5	168.0
2	143.8	179.2	130.7	157.0
3	216.3	234.1	189.9	238.9
4	205.5	160.8	149.7	186.8
5	156.0	131.2	103.6	135.9
6	189.8	207.2	167.5	245.1
7	168.4	156.7	141.4	160.8
8	169.9	139.5	135.7	109.7
9	231.7	227.3	169.4	296.9
10	196.0	175.1	167.7	191.8
Mean (µg/ml)	**185.7**	**179.5**	**150.4**	**186.1**
Mean Plasma/Serum (%)	**-**	**96.7**	**81.0**	**100.2**
Coefficient of determination R^2	**-**	**0.70**	**0.82**	**0.82**

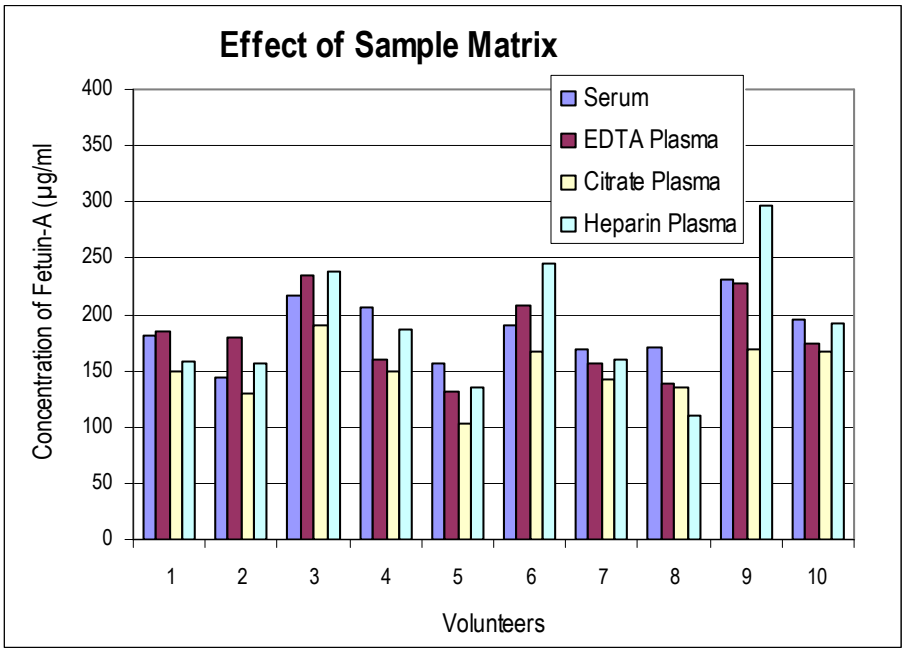

Figure 3: Fetuin-A concentrations measured at 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

- **Stability of samples stored at 2-8°C**

Samples should be stored at –20°C. However, no decline in concentration of fetuin-A was observed in serum and plasma samples after 14 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	*Incubation Temp, Period*	*Serum (µg/ml)*	*Plasma (µg/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	335.8	293.0	263.3	298.1
	2-8°C, 7 days	296.5	302.5	249.2	299.9
	2-8°C, 14 days	305.5	290.7	254.4	300.9
2	-20°C	347.9	312.2	271.1	377.2
	2-8°C, 7 days	329.7	326.2	288.5	438.9
	2-8°C, 14 days	315.4	289.7	270.1	386.3
3	-20°C	247.4	272.2	190.1	246.8
	2-8°C, 7 days	237.9	248.6	182.1	228.4
	2-8°C, 14 days	262.8	232.4	187.1	259.2

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human fetuin-A in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	*Number of f/t cycles*	*Serum (µg/ml)*	*Plasma (µg/ml)*		
			EDTA	*Citrate*	*Heparin*
1	1x	212.5	224.2	180.7	224.8
	3x	230.1	225.0	205.7	309.8
	5x	249.3	230.5	238.6	222.9
2	1x	459.0	297.5	277.0	289.4
	3x	499.8	364.7	286.2	463.5
	5x	488.1	263.2	299.4	318.0
3	1x	292.5	272.7	223.0	243.7
	3x	297.2	259.0	270.0	250.0
	5x	242.6	290.0	269.9	276.7

14. DEFINITION OF THE STANDARD

The Standard used in this kit is a natural Alpha 2 HS Glycoprotein (MW 49 kDa), isolated from human blood.

15. PRELIMINARY POPULATION AND CLINICAL DATA

Sera from twelve patients on long-term dialysis were measured and their fetuin-A concentrations were compared to control sera of seven pregnant woman (expected healthy) and to a pooled serum from normal, apparently healthy, individuals:

Patients – sample number	*Concentration of Fetuin-A (µg/ml)*	*CV (%)*
1	147.0	6.5
2	171.4	3.6
3	107.8	8.1
4	123.4	5.1
5	175.5	3.4
6	167.4	8.6
7	145.0	5.7
8	154.3	5.9
9	152.3	5.3
10	175.2	5.1
11	178.6	1.9
12	125.5	3.5

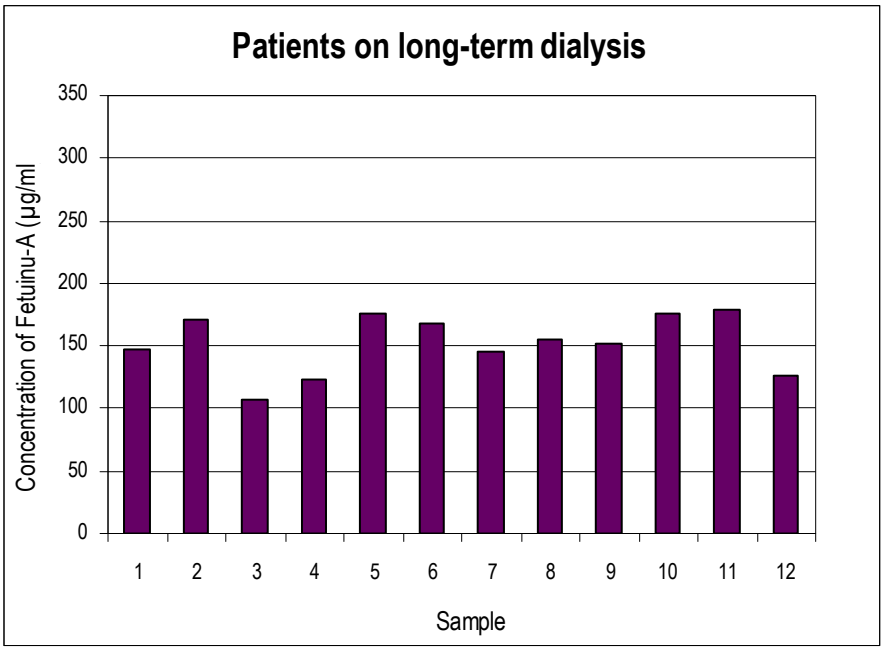

Figure 4: Fetuin-A concentration was determined in serum samples of twelve patients on long-term dialysis.

Control Samples	*Concentration of Fetuin-A (µg/ml)*	*CV (%)*
Pooled serum	256.2	11.2
1	248.9	2.3
2	200.4	5.2
3	231.5	4.3
4	314.5	3.8
5	179.9	1.6
6	217.5	0.2
7	247.2	6.6

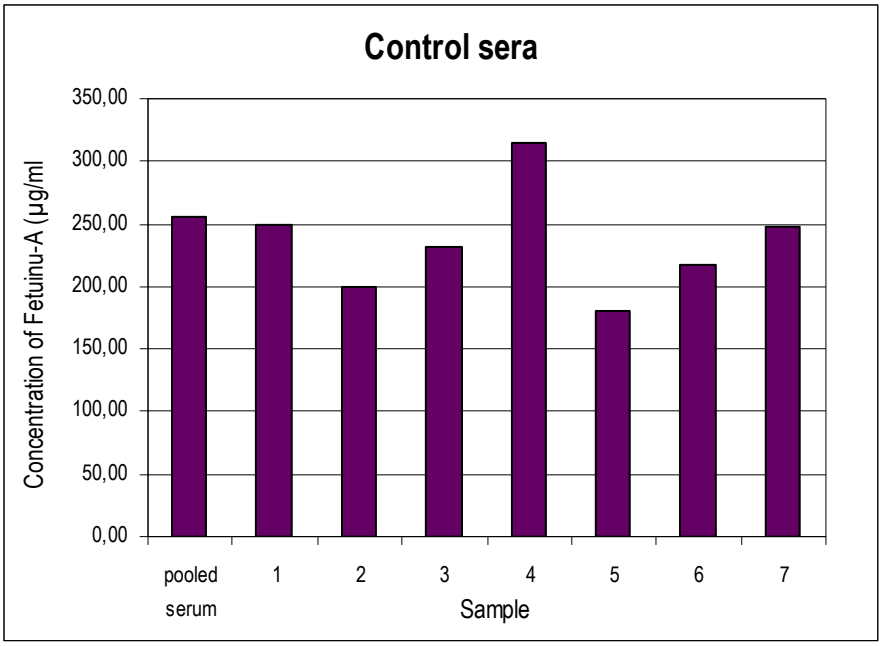

Figure 5: Pooled serum and samples of seven pregnant women (expected healthy) were used as control sera.

- **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 sample in the assay. Each laboratory should establish its own normal and pathological references ranges for fetuin-A levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Fetuin-A ELISA was compared to another commercial ELISA by measuring 33 serum samples. The following correlation graph was obtained:

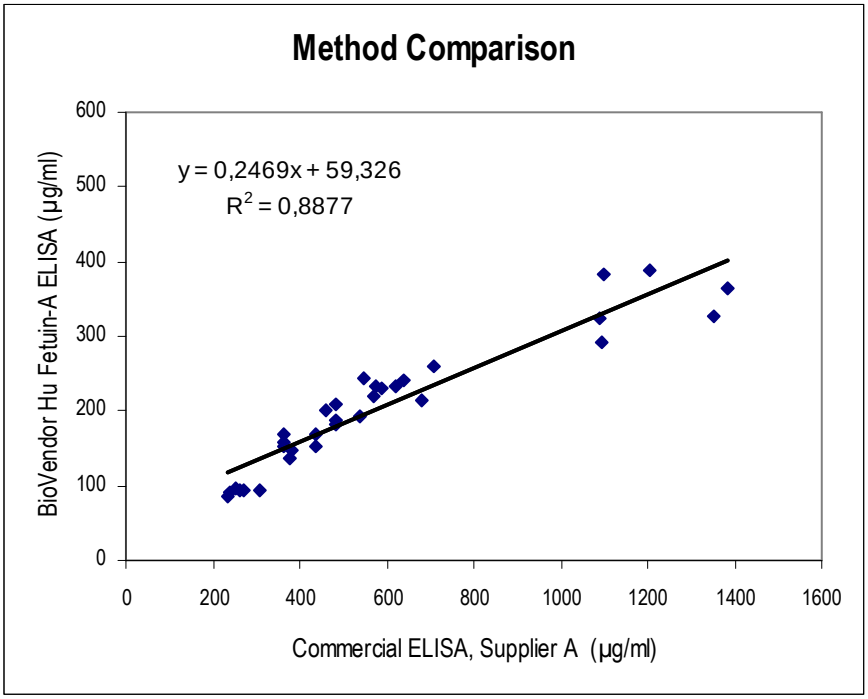

Figure 6: Method comparison.

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, Quality Controls or samples

18. REFERENCES

References to Fetuin-A:

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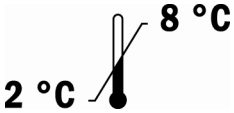
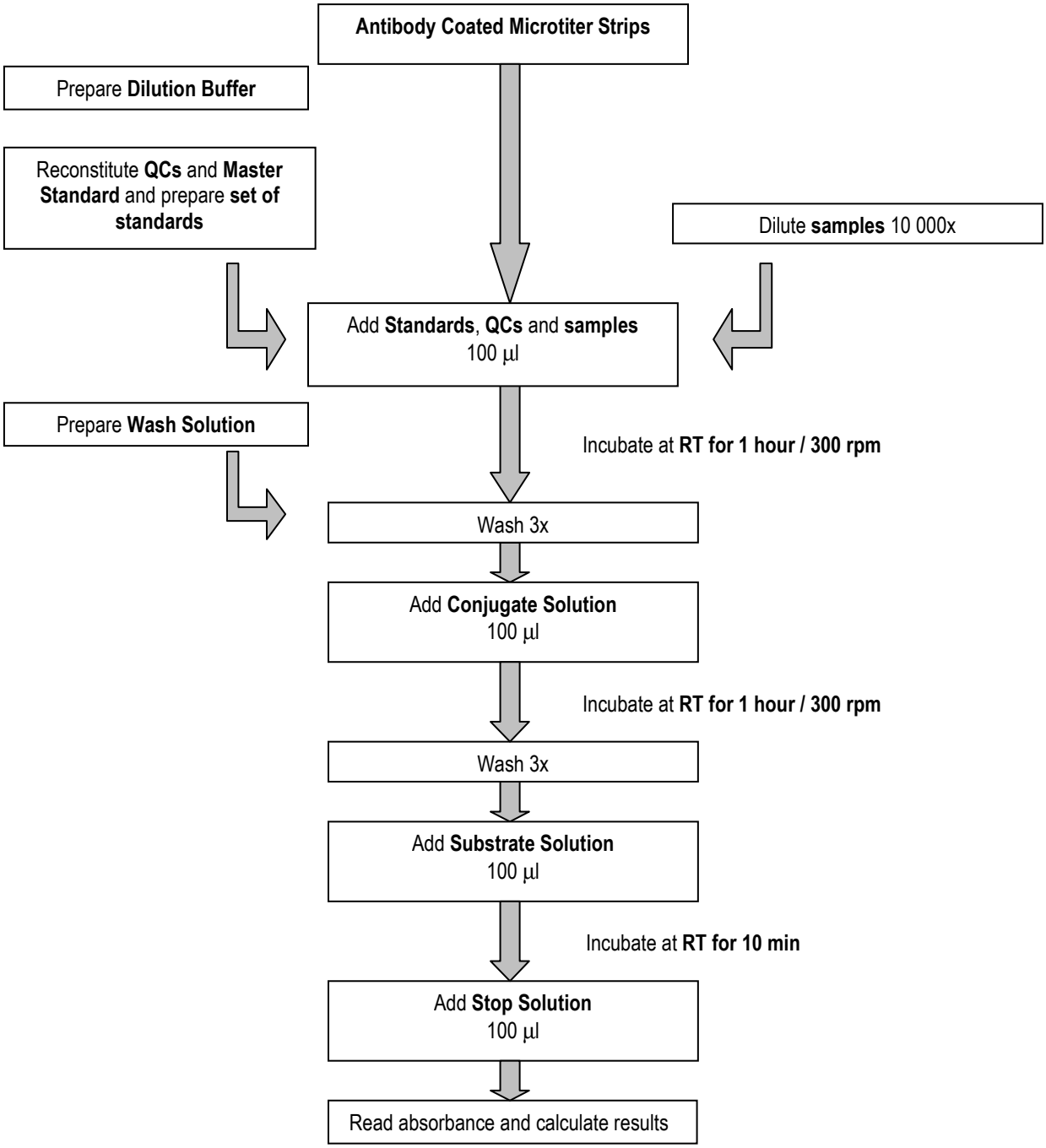
›› For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials
IVD CE	In vitro diagnostic medical device

Assay Procedure Summary



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NOTES

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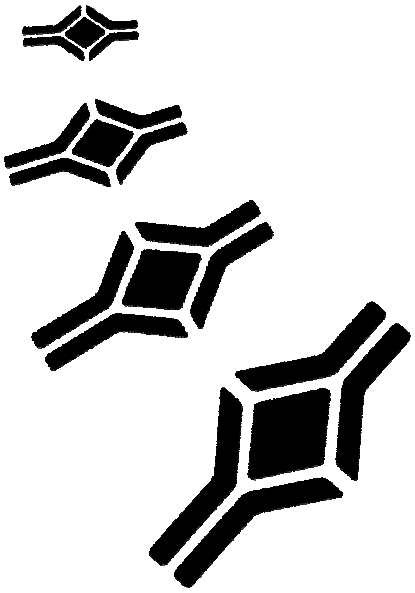

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