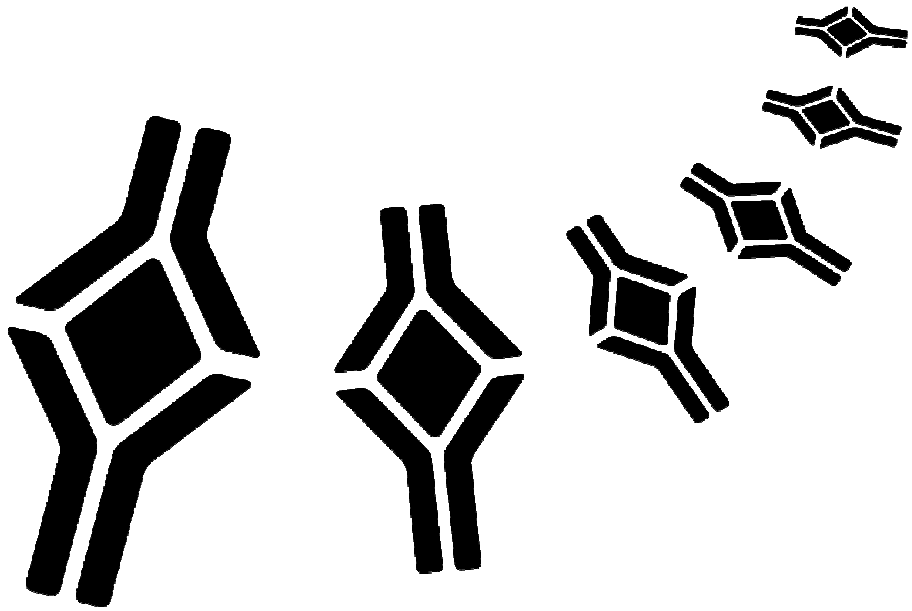
BioVendor
Research
and Diagnostic Products



HUMAN OSTEOACTIVIN ELISA

Product Data Sheet

Cat. No.: RD191170200R

For Research Use Only

CONTENTS

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 RD191170200R Human Osteoactivin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of Osteoactivin.

Features

- **It is intended for research use only**
- The total assay time is less than 3 hours
- The kit measures Osteoactivin protein 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

Osteoactivin (OA), also known as Dchil (dendritic cell-associated, heparin sulfate proteoglycan-dependent integrin ligand), Gpnmb (glycoprotein non-metastatic melanomal protein B), or Hgfin (hematopoietic growth factor-inducible neurokinin 1), is a transmembrane glycoprotein. The OA gene, located on human chromosome 7p15.1 encodes a protein of 572 amino acid residues. OA may exist as a 65-kD unglycosylated cellular protein or as multiple glycosylated proteins with molecular size varying between 80-kD to 139-kD. Glycosylation of proteins plays a crucial role in cell differentiation and function. The transmembrane OA can be proteolytically cleaved by extracellular proteases, such as ADAMs and MMPs, in a process called ectodomain shedding, which results in the detachment and release of the extracellular domains which act as cytokines or growth factors.

OA is expressed in a wide array of tissues and plays a regulatory role in various cellular functions. OA expression is associated with cell differentiation with high expression levels of OA protein found in the nervous system, basal layer of the skin, germinal cells of hair follicles, and in developing nephrons of the kidney in mouse embryos. In immune cells, OA was detected in differentiated macrophages, lymphocytes, and dendritic cells, but undetectable in proliferating hematopoietic progenitors.

It was reported that osteoblast-derived OA has a regulatory role in osteoblast differentiation and bone formation. OA expression in osteoblasts is up-regulated by bone morphogenetic protein-2 (BMP-2) and OA appears to be a key mediator of BMP-2-induced osteoblast differentiation.

Because of its suggested functions in cell adhesion, migration, and differentiation in various cell types and tissues, OA has been implicated in physiological and pathophysiological cascades of tissue injury and repair.

In addition to its diverse roles in normal cells and tissues, aberrant OA expression is linked to various pathological disorders such as glaucoma, kidney disease, osteoarthritis, amyotrophic lateral sclerosis and several types of cancer, including: uveal melanoma, glioma, hepatocellular carcinoma, and cutaneous melanoma. The discovery that osteoactivin is selectively overexpressed in aggressive bone metastatic breast cancer cells suggests an important role for this molecule in the progression to metastatic breast cancer.

<u>Areas of investigation:</u>

- Bone/skeletal muscles degeneration/regeneration
- Oncology (glioma, hepatocelular carcinoma, bone metastatic breast cancer)
- Neural tissue
- Renal disease

4. TEST PRINCIPLE

In the BioVendor Human Osteoactivin ELISA, the standards and samples are incubated in microtitrate wells pre-coated with polyclonal anti-human Osteoactivin antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human Osteoactivin antibody is added and incubated with the captured Osteoactivin 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 Osteoactivin. 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 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

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc.	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Biotin-Ab Diluent	ready to use	1 x 13 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 15-1000 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 550-650 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.

Dilution Buffer
Biotin-Ab Diluent
Streptavidin-HRP Conjugate
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 Osteoactivin 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 Osteoactivin in the stock solution is **50 ng/ml.**

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	50 ng/ml
300 µl of stock	300 µl	25 ng/ml
300 µl of 25 ng/ml	300 µl	12.5 ng/ml
300 µl of 12.5 ng/ml	300 µl	6.25 ng/ml
300 µl of 6.25 ng/ml	300 µl	3.13 ng/ml
300 µl of 3.13 ng/ml	300 µl	1.56 ng/ml
300 µl of 1.56 ng/ml	300 µl	0.78 ng/ml

Prepared Standards are ready to use, do not dilute them.
Stability and storage:
The reconstituted Master Standard must be used immediately.
Do not store the Standard stock solutions and set of standards.

Biotin Labelled Antibody
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of Biotin Labelled Antibody!!!
Reconstitute the lyophilized Biotin Labelled Antibody with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Dilute reconstituted Biotin Labelled Antibody Concentrate (100x) with Conjugate Diluent e.g. 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells).
Stability and storage:
Biotin Labelled Antibody Concentrate (100x) is stable 1 month 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 Osteoactivin 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 serum or plasma samples 10x with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 135 µl of Dilution Buffer when assaying samples as singlets or preferably 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **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.

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 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. (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). No shaking!
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: 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 Osteoactivin 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 50**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 25**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 12.5**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 6.25**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 3.13**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 1.56**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 0.78**	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 microtiter plate readers perform automatic calculations of analyte concentration. The Standards curve is constructed by plotting the 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 Osteoactivin (ng/ml) in samples.

Alternatively, the *logit log* function can be used to linearize the standard curve, i.e. *logit* of 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. 5 ng/ml (from standard curve) x 10 (dilution factor) = 50 ng/ml.

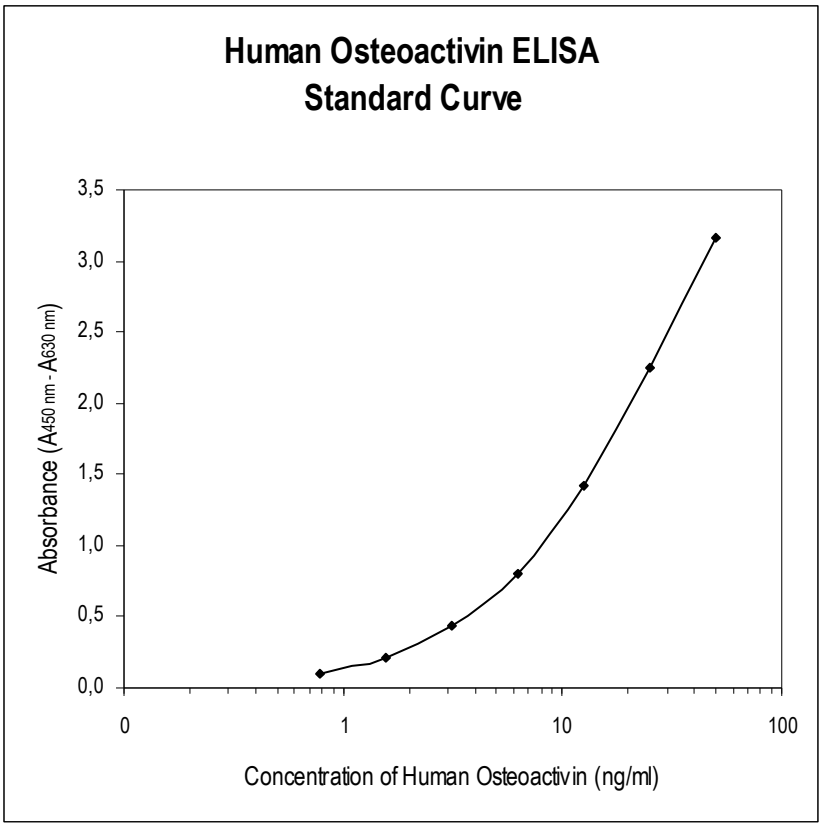


Figure 2: Typical Standard Curve for Human Osteoactivin ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Osteoactivin 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 Osteoactivin values in wells and is 0.102 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.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	56.8	1.6	2.8
2	77.6	2.2	2.8

Inter-assay (Run-to-Run) (n=5)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	30.5	1.9	6.4
2	52.3	3.0	5.7

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Osteoactivin and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	73.3	-	-
	100.0	97.0	103.2
	128.7	123.8	104.0
	184.4	177.3	104.0
2	46.6	-	-
	72.7	70.3	103.4
	99.7	97.1	102.7
	159.3	150.7	105.7

- **Linearity**

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	63.2	-	-
	2x	31.0	31.6	98.0
	4x	14.7	15.8	92.9
	8x	7.4	7.9	93.8
2	-	98.2	-	-
	2x	49.8	49.1	101.4
	4x	23.9	24.5	97.2
	8x	11.1	12.3	90.5

- **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	78.3	84.6	68.7	80.8
2	59.0	62.1	53.7	56.0
3	27.0	27.6	25.4	27.7
4	40.2	39.5	34.0	37.8
5	40.7	43.3	35.2	42.1
6	41.8	52.3	37.4	43.1
7	50.1	52.3	42.6	48.8
8	39.8	55.2	38.7	37.8
9	35.1	41.2	34.3	35.3
10	34.9	44.0	34.2	35.4
Mean (ng/ml)	**44.7**	**50.2**	**40.4**	**44.5**
Mean Plasma/Serum (%)		**112.3%**	**90.4%**	**99.5%**
Coefficient of determination R^2		**0.90**	**0.98**	**0.99**

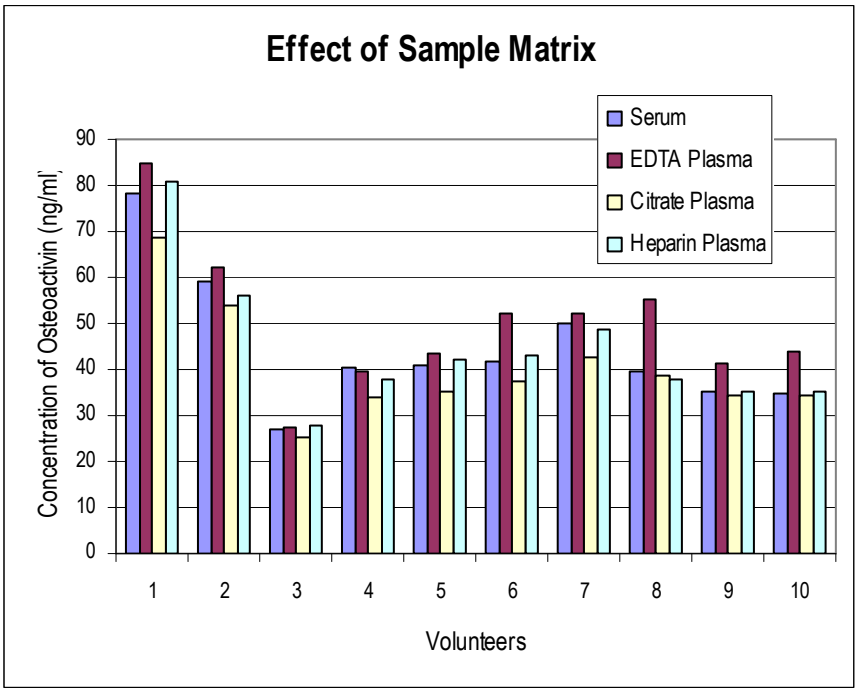


Figure 3: Osteoactivin levels measured using Human Osteoactivin ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. PRELIMINARY POPULATION DATA

The following results were obtained when serum samples from 160 unselected donors (88 men + 72 women) 21-65 years old were assayed with the Biovendor Human Osteoactivin ELISA in our laboratory.
Each laboratory should establish its own normal and pathological references ranges for Osteoactivin levels with the assay.

Sex	*Age (years)*	*n*	*Mean*	*Median*	*SD*	*Min*	*Max*
			Osteoactivin (ng/ml)				
Men	21-29	17	44.1	41.1	10.2	29.0	62.6
	30-39	27	43.8	44.7	7.0	30.7	61.4
	40-49	32	60.6	46.4	46.9	31.7	297.7
	50-65	12	48.5	41.8	29.4	30.5	140.6
Women	22-29	13	58.1	40.7	38.9	34.4	169.3
	30-39	28	38.0	28.3	11.3	22.8	79.4
	40-49	23	38.0	35.8	13.9	25.3	92.9
	50-61	8	46.3	41.8	16.1	31.0	81.2

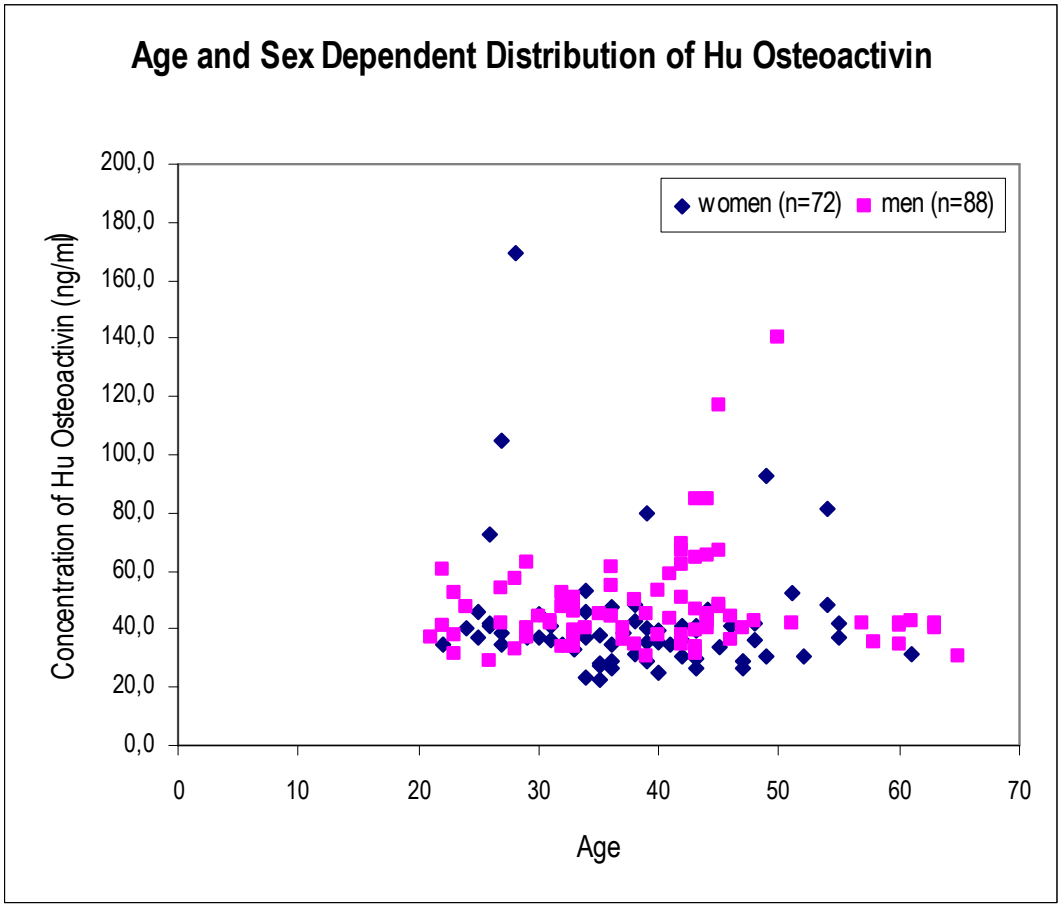


Figure 4: Human Osteoactivin concentration plotted against donor age.

15. DEFINITION OF THE STANDARD

The recombinant human Osteoactivin is used as the Standard. The recombinant human Osteoactivin, produced in *HEK293*, is 53.7 kDa protein containing 477 amino acid residues of the human Osteoactivin and 10 additional amino acid residues- His Tag.

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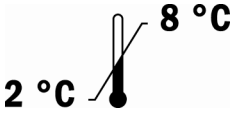
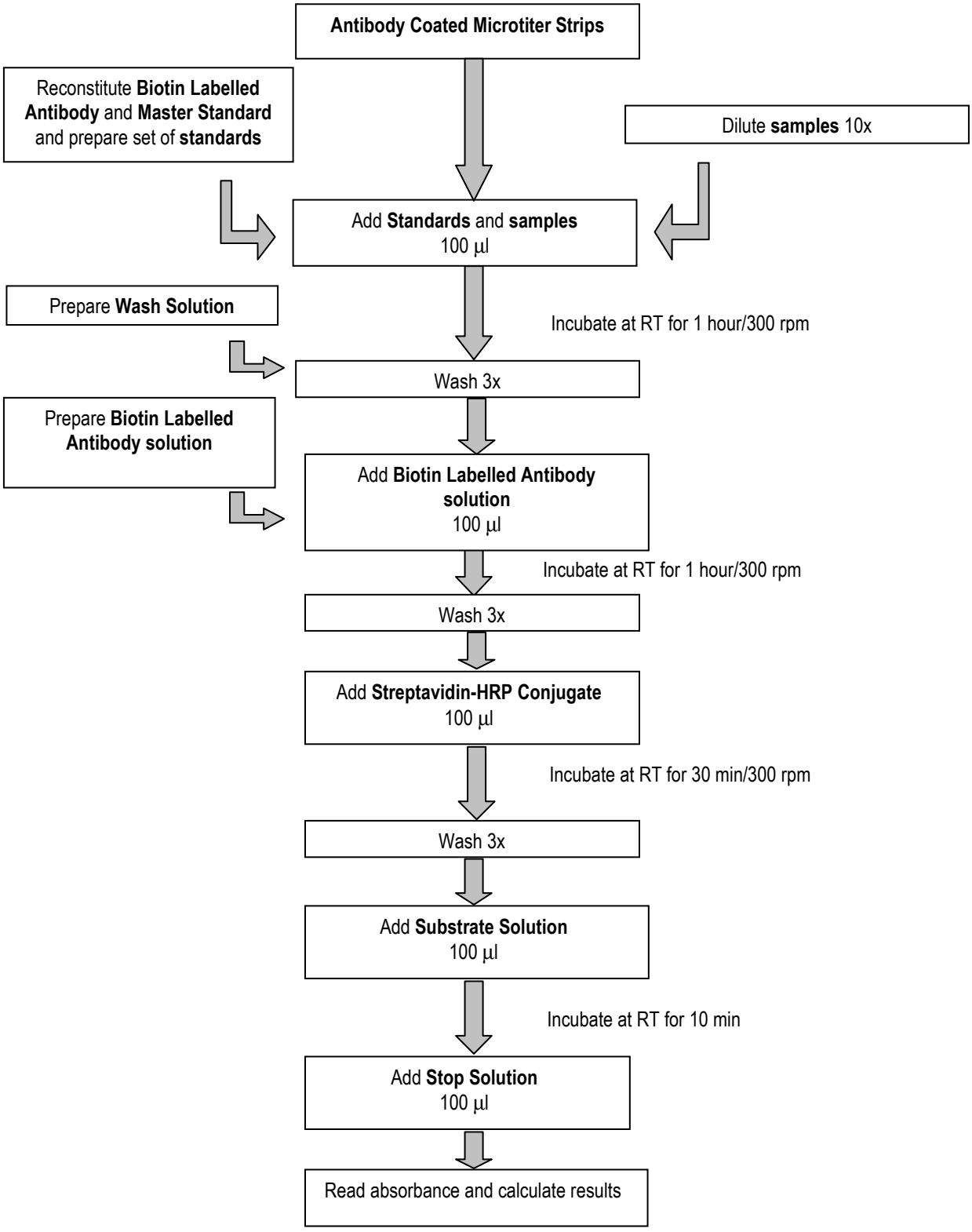
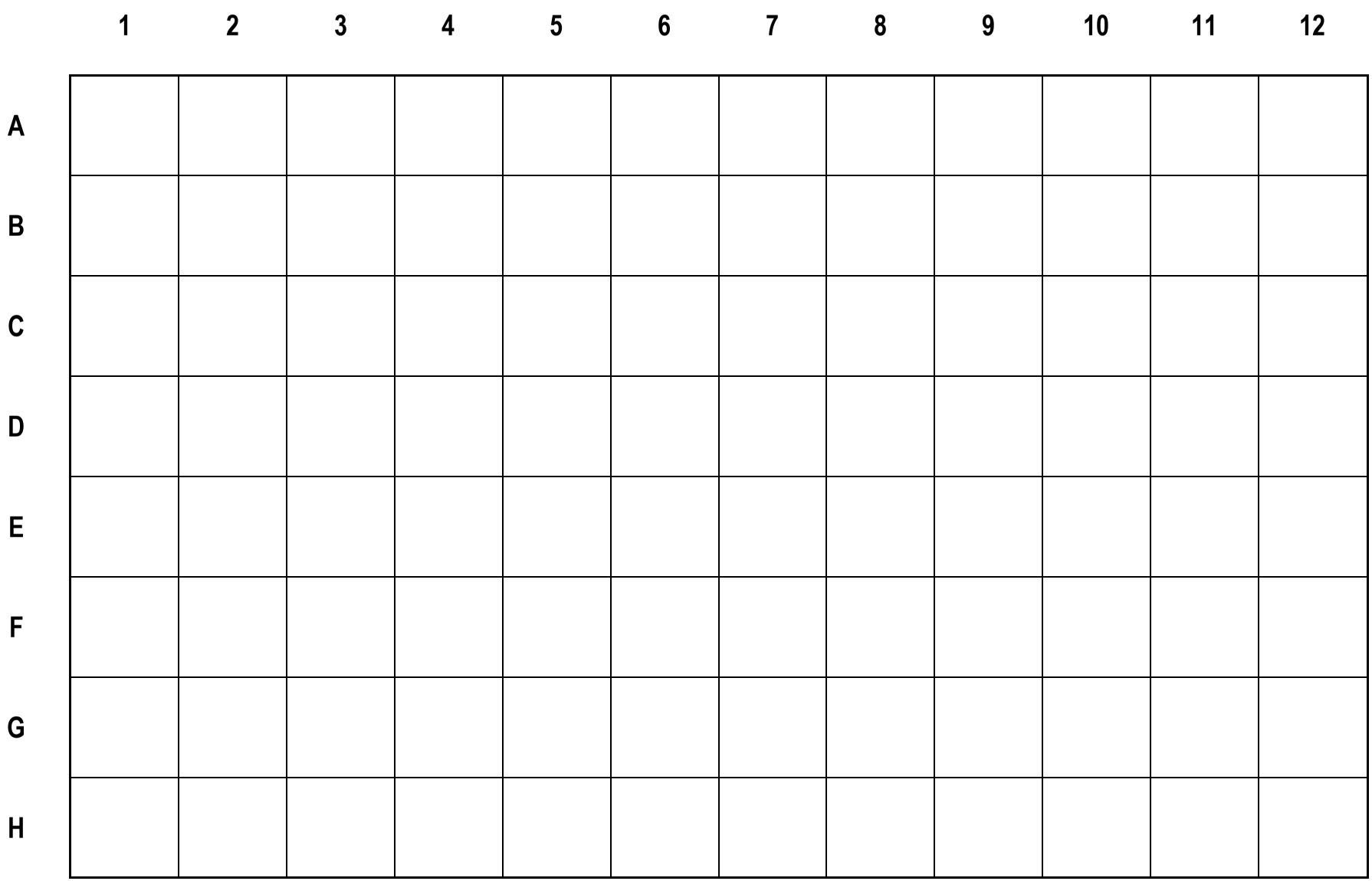
» **For more references on this product see our WebPages at www.biovendor.com**

18. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Expiry date
	Storage conditions
	Identification of packaging materials

Assay Procedure Summary

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19. NOTES

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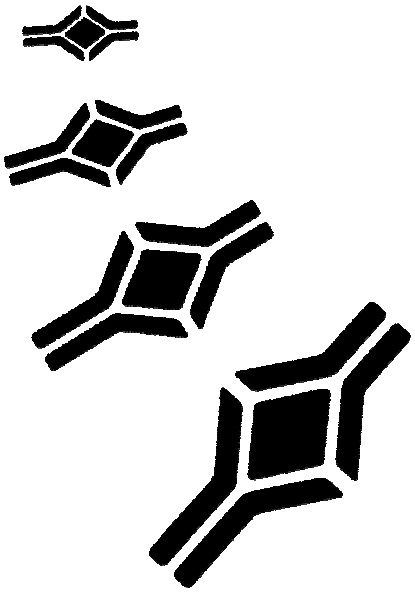
BioVendor
Research
and Diagnostic Products



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