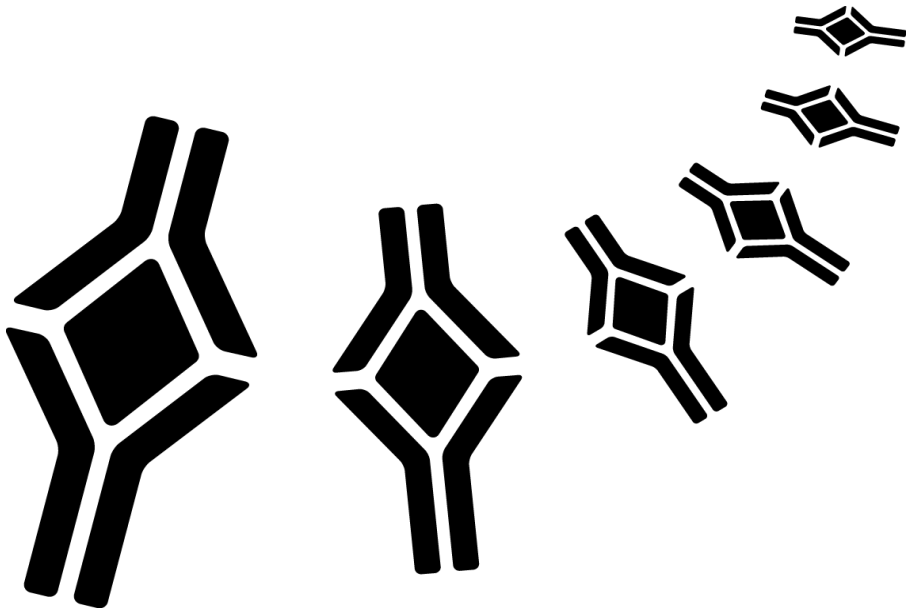
BioVendor
Research
and Diagnostic Products



HUMAN OSTEOPONTIN ELISA

Product Data Sheet

Cat. No.: RD191446200R

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 RD191446200R Human Osteopontin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human osteopontin.

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures osteopontin in plasma (EDTA, citrate, heparin), serum, urine cerebrospinal fluid (CSF) and breast milk
- 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

Osteopontin (OPN) also named secreted phosphoprotein-1 (SPP1) and sialoprotein-1, is negatively charged aspartic acid-rich, N-linked glycosylated phosphoprotein composed of 314 amino acid residues. OPN exists in various isoforms as a result of alternative splicing, alternative translation and different posttranslation modifications, which result in different molecular weights ranging from 41 to 75 kDa. OPN has primarily been described as a secreted protein but additional evidence suggested that it can also be found in the cytoplasma and nucleus. This form of intracellular OPN (iOPN) is the result of alternative translation and has biological functions distinct from those of secreted OPN (sOPN). OPN molecule has an arginine-glycine-aspartic acid (RGD) cell binding sequence, a calcium binding site and two heparin binding domains. OPN can be modified by thrombin cleavage which exposes additional cryptic integrin-binding sites. Cells bind OPN via multiple cell surface receptors, including various integrin receptors and CD44.[2,7,15,18]

OPN is highly expressed in bone (osteoclasts and osteoblasts) and also secreted by various cell types including macrophages, activated T lymphocytes, endothelial cells, smooth muscle cells, epithelial cells, inner ear, brain, placenta and mammary glands, decidua and kidney. Secreted OPN is found in various biological fluids including blood, milk, urine, cerebrospinal fluid, synovial fluid and seminal fluid. OPN is involved in both physiological and pathophysiological processes in multiple organs and tissues.[2,18]

One major physiological function of sOPN is the control of biomineralization. As a member of SIBLING protein family with overall negative charge, OPN is able to directly bind to specific apatite crystal faces thereby acts as a mineralization inhibitor. OPN is also strongly upregulated at sites of ectopic, pathologic calcification – such as vascular calcification, valvular calcification, renal crystal formation and gallstone formation and prevents or limits calcification. Moreover OPN is required for bone remodeling process and stimulates adhesion, migration and bone resorption by osteoclast.[2,18,19]

Abundant evidence suggests that OPN plays a critical role in acute inflammation and leukocyte recruitment and in chronic inflammatory diseases such as multiple sclerosis, Crohn´s disease and other autoimmune disorders, several types of cancer and cardiovascular diseases. OPN may exert both pro-inflammatory and anti-inflammatory actions depending on biological requirement. Increased OPN levels in cerebrospinal fluid have been found in patients with inflammatory neurological disease (e.g. multiple sclerosis, Alzheimer´s disease and neuromyelitis optica) and may reflect disease progression.[5,8,9,11,13,17,22]

OPN is a critical regulator of adipose tissue inflammation in obesity. In adipose tissue, OPN is upregulated, induces infiltration and activation of macrophages and these infiltrated macrophages produce proinflammatory cytokines which contribute to adipose tissue insulin resistance and type 2 diabetes. Furthermore, OPN was shown to negatively influence atherosclerosis and hepatic disorders which are strongly associated with obesity and type 2 diabetes such as non-alcoholic fatty liver disease (NAFLD) and diabetic nephropathy. Clinical approaches show that circulating OPN levels in obese patients were elevated compared with lean subjects and were further increased in obese diabetic or insulin resistant patients.[6,10,16,18,20]

OPN is a tumor-associated antigen that is highly expressed in multiple human cancers including lung cancer, breast cancer, melanoma and mesothelioma. The level of circulating OPN may be indicative of cancer progression, metastasis, and prognosis. Recent study shows, that OPN plasma level of metastatic breast cancer patients is significantly higher in comparison with a non-metastatic group, and OPN can be a biochemical marker giving early signal for metastases.[3,4,24]
Urinary OPN concentration can be used for investigation of renal stone disease because OPN inhibits urinary crystallization.
It has been reported that elevated OPN level was presented in plasma and synovial fluid of patients with rheumatoid arthritis (RA) and with osteoarthritis (OA) compared to the control OPN level. Measurements of plasma and synovial fluid levels of OPN in patients with primary knee OA reveal significant correlation with severity of knee pain and radiologic progression of the disease.[14,21,23]
Effect of OPN for cell survival was demonstrated on epithelial, endothelial and smooth muscle cells. The experiment indicated that the OPN binding to $\alpha_v\beta_3$ integrin of endothelial cells activates the pro-survival transcription factor NFκB and protects cells from undergoing apoptosis.[1,2]
Current clinical investigation of critically ill patients with and without sepsis described that persistently elevated OPN serum concentrations are associated with unfavorable outcome independent of the presence of sepsis. Studies indicate that OPN may be a prognostic biomarker in these patients during early course of treatment in medical intensive care units.[25]

Areas of investigation:
Bone and cartilage metabolism
Cardiovascular disease
Diabetes
Inflammation
Neurodegenerative disease
Obesity
Oncology
Renal disease

4. TEST PRINCIPLE

In the BioVendor Human Osteopontin ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human osteopontin antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human osteopontin antibody is added and incubated for 60 minutes with captured osteopontin. 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 osteopontin. 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 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
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 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 desicant and seal carefully. Remaining Microtiter Strips are stable 3 months 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 Osteopontin 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 osteopontin in the stock solution is **16 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	16 ng/ml
250 µl of stock	250 µl	8 ng/ml
250 µl of 8 ng/ml	250 µl	4 ng/ml
250 µl of 4 ng/ml	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

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:
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 osteopontin in plasma (EDTA, citrate, heparin), serum, urine, cerebrospinal fluid (CSF) and breast milk.

Osteopontin is proteolytically cleaved by thrombin during blood clotting, thus, serum osteopontin levels are significantly lower than plasma levels. To prevent OPN cleavage in samples during clotting, PMSF (phenylmethylsulfonyl fluoride - serine protease inhibitor) in final concentration of 1 mM can be added. However, we obtained a good correlation between serum and plasma OPN levels measured with the kit – please see the chapter 13. Performance Characteristics (Effect of Sample Matrix).

Samples should be assayed immediately after collection or should be stored at -20°C or preferably at -70°C. **Storage of samples at 4°C should be avoided since it leads to a significant decrease in OPN levels.** 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.

Plasma samples:
Dilute samples 10x with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets, or preferably 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Serum samples:
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 is 100x.

Recommended starting dilution for CSF is 20x.

Recommended starting dilution for breast milk is 2000x.

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.

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 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 5-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 5-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 5-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 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 osteopontin 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 four times. 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 16**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 8**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 4**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 2**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 1**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 0.5**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 0.25**	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 osteopontin (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 have been diluted prior to the assay, e.g. 2 ng/ml (from standard curve) x 10 (dilution factor) = 20 ng/ml

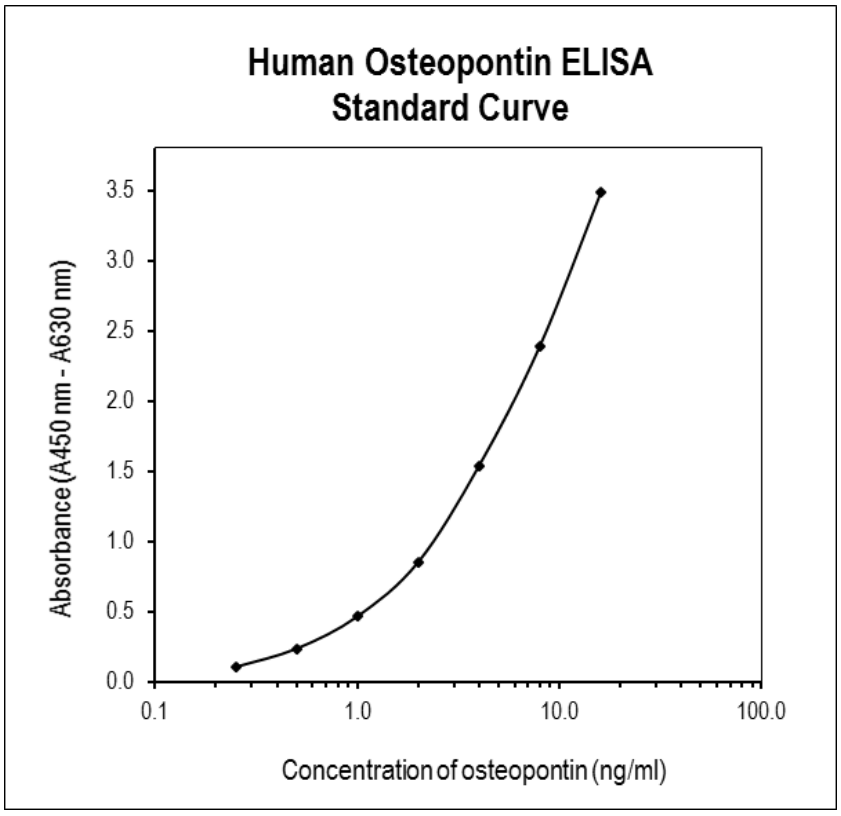


Figure 2: Typical Standard Curve for Human Osteopontin ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human Osteopontin 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 osteopontin values in wells and is 87 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.

» Presented results are multiplied by respective dilution factor

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	12.82	0.61	4.7
2	17.46	1.15	6.6

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	14.73	0.67	4.5
2	24.14	0.77	3.2

- **Spiking Recovery**

Samples were spiked with different amounts of human osteopontin and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
EDTA plasma 1	25.94 41.64 54.09 84.47	- 40.94 55.94 85.94	- 101.7 96.7 98.3
EDTA plasma 2	24.77 41.39 54.74 70.02	- 39.77 54.77 84.77	- 104.1 99.9 82.6
Serum 1	5.98 9.95 13.69 21.70	- 10.98 15.98 25.98	- 90.6 85.6 83.5
Serum 2	7.45 13.05 16.22 22.95	- 12.45 17.45 27.45	- 104.9 93.0 83.6

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
EDTA plasma 1	- 2x 4x 8x	23.66 11.88 5.49 2.56	- 11.83 5.92 2.96	- 100.4 92.8 86.6
EDTA plasma 2	- 2x 4x 8x	28.22 14.76 7.28 3.69	- 14.11 7.06 3.53	- 104.6 103.2 104.6
Serum 1	- 2x 4x 8x	11.37 5.88 2.96 1.58	- 5.69 2.84 1.42	- 103.5 104.2 111.0
Serum 2	- 2x 4x 8x	21.65 10.12 4.73 2.63	- 10.83 5.41 2.71	- 93.5 87.3 97.1

- **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 (ng/ml)*	*Plasma (ng/ml)*		
		EDTA	*Citrate*	*Heparin*
1	12.45	23.33	17.58	25.54
2	20.05	27.54	20.93	32.96
3	14.56	27.44	18.62	26.08
4	19.82	32.15	27.58	33.67
5	12.23	22.59	18.26	30.02
6	9.35	14.48	11.30	13.89
7	18.04	29.41	22.85	30.68
8	11.77	22.84	18.40	20.47
9	16.42	28.40	24.32	34.68
10	7.41	11.46	11.48	14.08
Mean (ng/ml)	**14.21**	**23.96**	**19.13**	**26.21**
Mean Plasma/Serum (%)	**-**	**168.7**	**134.7**	**184.5**
Coefficient of determination R^2	**-**	**0.85**	**0.80**	**0.79**

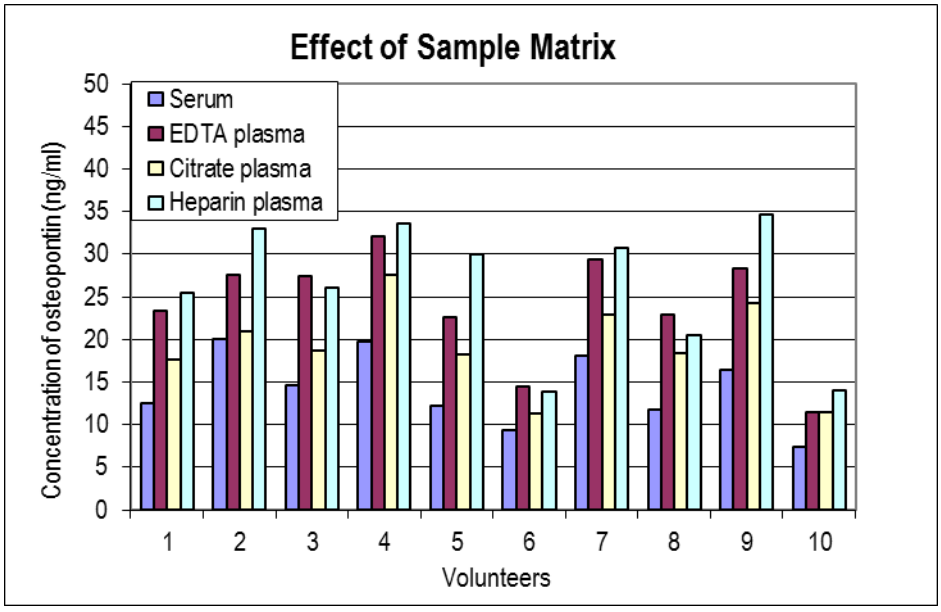


Figure 3: Osteopontin levels measured using Human Osteopontin ELISA in serum, EDTA, citrate and heparin plasma, respectively, from the same 10 individuals.

14. DEFINITION OF THE STANDARD

Recombinant human osteopontin is used as the standard. The recombinant human osteopontin produced in HEK293 cells is 33.7 kDa protein containing 298 amino acid residues.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 160 unselected donors (89 men + 71 women) 21-65 years old were assayed with the BioVendor Human Osteopontin ELISA in our laboratory.

- **Age dependent distribution of osteopontin**

Sex	*Age (years)*	*n*	*Osteopontin (ng/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	21-29	18	11.77	10.83	5.30	3.67	21.95
	30-39	28	12.16	11.13	5.45	3.70	29.24
	40-49	32	12.31	11.49	5.38	2.55	27.03
	50-65	11	12.16	12.81	3.73	6.08	20.45
Women	22-29	13	11.12	9.31	6.73	1.33	23.77
	30-39	28	10.26	10.20	4.30	3.64	19.70
	40-49	22	10.02	9.02	4.83	2.68	25.14
	50-61	8	10.46	9.52	5.20	3.82	20.49

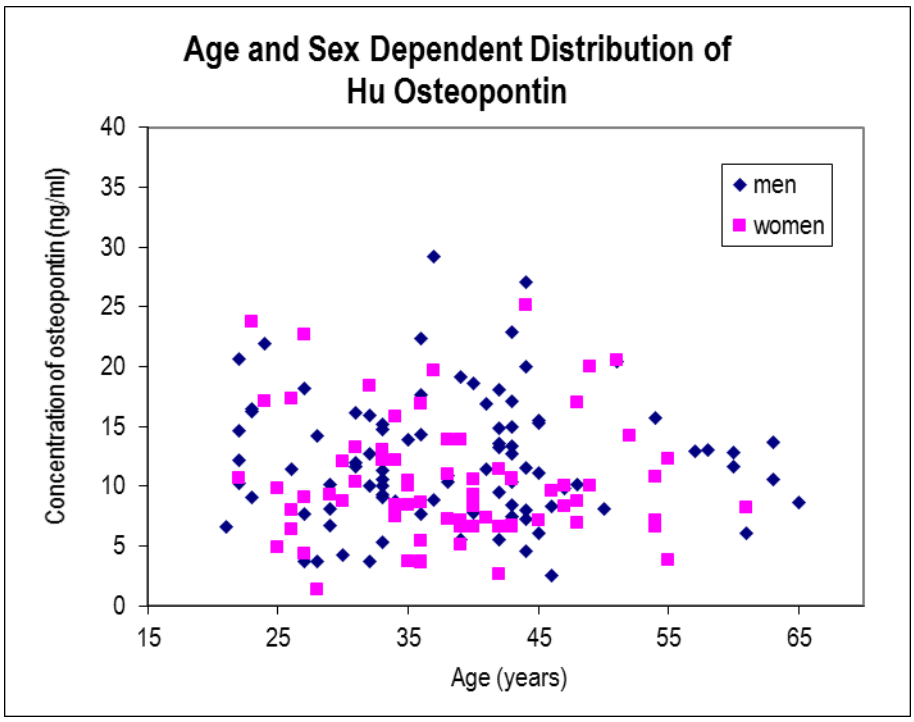


Figure 4: Human osteopontin concentration plotted against donor age and sex.

- **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 osteopontin levels with the assay.

16. METHOD COMPARISON

The BioVendor Human Osteopontin ELISA was compared to another commercial immunoassay by measuring 16 EDTA plasma samples and 20 serum samples. The following correlation graph was obtained:

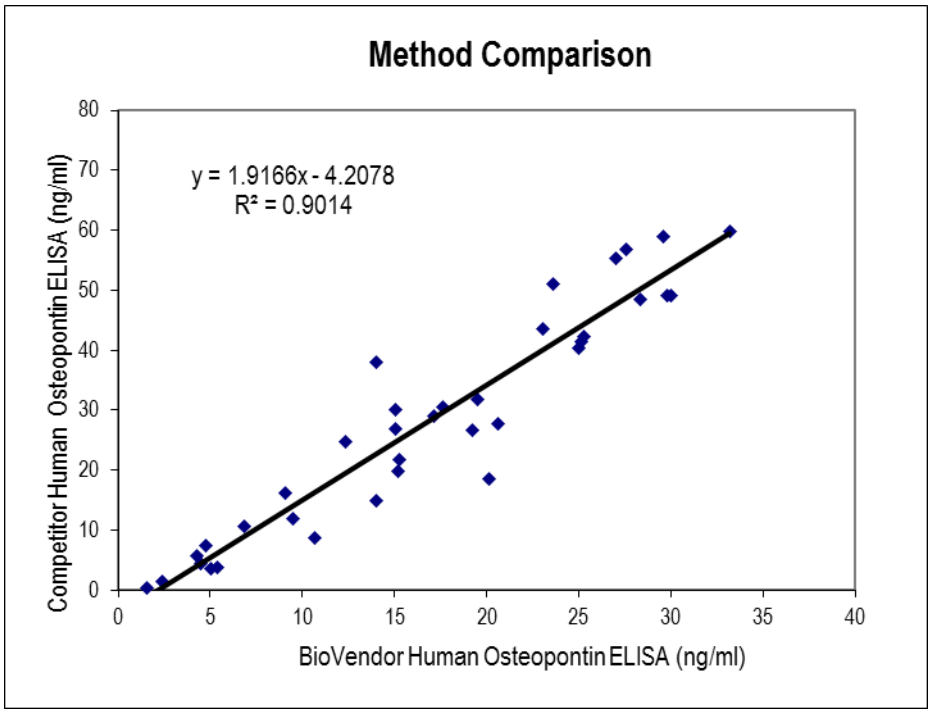


Figure 5: Method Comparison

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

›› High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards and samples

18. REFERENCES

References to osteopontin:

1. Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM: NF-κB mediates αvβ3 integrin-induced endothelial cell survival. J of Cell Biol 1998, 141(4):1083-1093
2. Mazzali M, Kipari T, Ophascharoensuk V, Wesson JA, Johnson R, Hughes J: Osteopontin – a molecule for all seasons. Q J Med 2002:95:3-13
3. El-Tanani MK, Campbell FC, Kurisetty V, jin D, McCann M, Rudland PS: The regulation and role of osteopontin in malignant transformation and cancer. Cytokine Growth Factor Rev 2006, 17(6):463-474
4. Bramwell VHC, Doig GS, Tuck AB, Wilson SM, Tonkin KS, Tomiak A, Perera F, Vanderberg TA, Chambers AF: Serial plasma osteopontin levels have prognostic value in metastatic breast cancer. Clin Cancer Res 2006, 12(11):3337-3343
5. Scatena M, Liaw L, Giachelli CM: Osteopontin: a multifunctional molecule regulating chronic inflammation and vascular disease. Arterioscle Throm and Vas Biol 2007, 27:2302-2309
6. Kiefer FW, Zeyda M, Todoric J, Huber J, Geyeregger R, Weichhart T, Aszmann O, Ludvik B, Silberhumer GR, Prager G, Stulnig TM: Osteopontin expression in human and murine obesity: Extensive local up-regulation in adipose tissue but minimal systemic alterations. Endocrinology 2008, 149(3):1350-1357
7. Sinohara ML, Kim HJ, Kim JH, Garcia VA, cantor H: Alternative translation of osteopontin generates intracellular and secrete isoforms that mediate distinct biological activities in dendritic cells. PNAS 2008, 105(20):7235-7239
8. Rosenberg M, Zugck Ch, Nelles M, Juenger C, Frank D, Remppis A, Giannitsis E, Katus HA, Frey N: Osteopontin, a new prognostic biomarker in patients with chronic heart failure. Circ Heart Fail 2008, 1:43-49
9. Braitch M, Nunan R, Niepel G, Edwards LJ, Constantinescu CS: Increased osteopontin levels in the cerebrospinal fluid of patients with multiple sclerosis. Arch Neurol 2008, 65(5):633-635
10. Bertola A, Deveaux V, Bonnafous S, Rousseau D, Anty R, Wakkach A, Dahman M, Tordjman J, Clément K, McQuaid SE, Frayn KN, Huet PM, Gugenheim J, Lotersztajn S, Le Marchand-Brustel Y, Tran A, Gual P: Elevated expression of osteopontin may be related to adipose tissue macrophage accumulation and liver steatosis in morbid obesity. Diabetes 2009, 58:125-133
11. Lund SA, Giachelli CM, Scatena M: The role of osteopontin in inflammatory processes. J of Cell Comun and Signaling 2009, 3:311-322
12. Kiefer FW, Zeyda M, Gollinger K, Pfau B, Neuhofer A, Weichhart T, et al.: Neutralization of osteopontin inhibits obesity-indeced inflammation and insulin resistance. Diabetes 2010, 59:935-946
13. Comi C, Carecchio M, Chiocchetti A, Nicola S, Galimberti D, Fenoglio Ch, Cappellano G, Monaco F, Scarpini E, Dianzani U: Osteopontin is increased in the cerebrospinal

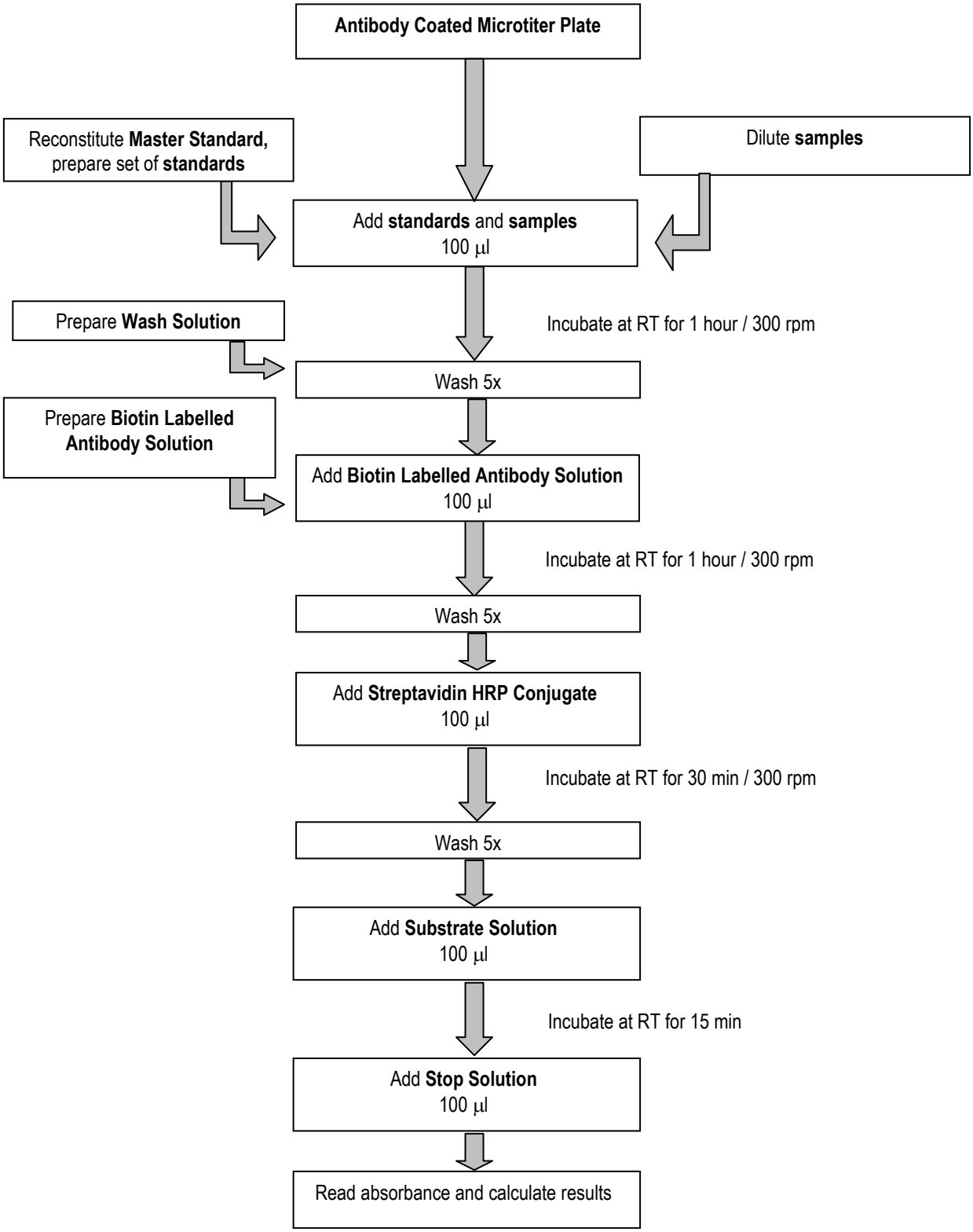
fluid of patients with Alzheimer´s disease and its levels correlate with cognitive decline. J of Alzh Dis 2010, 19:1143-1148
14. Gao SG, Li KH, Zeng KB, Tu M, Xu M, Lei GH: Elevated osteopontin level of synovial fluid and articular cartilage is associated with disease in knee osteoarthrithis patients. Osteoarthritis and Cartilage 2010, 18:82-87
15. Inoue M, Shinohara ML: Intracellular osteopontin (iOPN) and immunity. Immun Rasearch 2011, 46:160-172
16. Zeyda M, Gollinger K, Todoric J, Kiefer FW, Keck M, Aszmann O, Prager G, Zlabinger GJ, Petzelbauer P, Stulnig TM: Osteopontin is a activator of human adipose tissue macrophages and directly affects adipocyte function. Endocrinology 2011, 152(6):2219-2227
17. Patouraux S, Bonnafous S, Voican CS, Anty R, Saint-Paul MCh, Rosenthal-Allieri MA, Agostini H, Njike M, Barri-Ova N, Naveau S, Le Marchand-Brustel Y, Veillon P, Cales P, Perlemuter G, Tran A, Gual P: The osteopontin level in liver, adipose tissue and serum is correlated with fibrosis in patients with alcoholic liver disease. PLos ONE 2012, 7(4):e35612
18. Kahles F, Findeisen HM, Bruemmer D: Osteopontin: a novel regulator at the cross roads of inflammation, obesity and diabetes. Mol Metabolism 2014, 3:384-393Sukhatme VP, Chan B: Glycolitic cancer cells lacking osteopontin metabolize glucose to induce senescence. FEBS Letters 2012, 586:2389-2395
19. Cho EH, Cho KH, Lee HA, Kim SW: High serum osteopontin levels are associated with low bone mineral density in postmenopausal women. J Korea Med Sci 2013, 28:1496-1499
20. Ahmad R, Al-Mass A, Al-Ghawas D, Shareif N, Zghoul N, Melhen M, Hasan A, Al-Ghimlas F, Dermine S, Behbehani K: Interaction of osteopontin with IL-18 in obese individuals: Implications for insulin resistance. Plos One 2013, 8(5):e63944
21. Iwadate H, Kobayashi H, Kanno T, Asano T, Saito R, Sato S, Suzuki E, Watanabe H, Ohira H: Plasma osteopontin is correlated with bone resorption markers in rheumatoid arthritis patients. In J Rheu Diseases 2014, 17:50-56
22. Kariya Y, Kariya Y, Saito T, Nishiyama S, Honda T, Tanaka K, Yoshida M, Fujihara K, Hashimoto Y: Increased cerebrospinal fluid osteopontin levels and its involvement in macrophage infiltration in neuromyelitis optica. BBA clinical 2015, 3:126-134
23. Haider HM, Amin IR, Ahmad KA: Plasma and synovial osteopontin levels, are they associated with disease severity of primary knee osteoarthritis in Egyptian patients? The Egyptian Rheumatologist 2015, 37:29-34
24. Nassar HR, Namour AE, Shafik HE, El Sayed AS, Kamel SM, Moneer MM, Zakhary NI: Prognostic significance of plasma osteopontin level in breast cancer patients. HeSMO 2015, 6(1):27-32
25. Roderburg Ch, Benz F, Cardenas DV, Lutz M, Hippe HJ, Luedde T, Trautwein Ch, Frey N, Koch A, Tacke F, Luedde M: Persistently elevated osteopontin serum levels predict mortality in critically ill patients. Critical Care 2015, 19:271

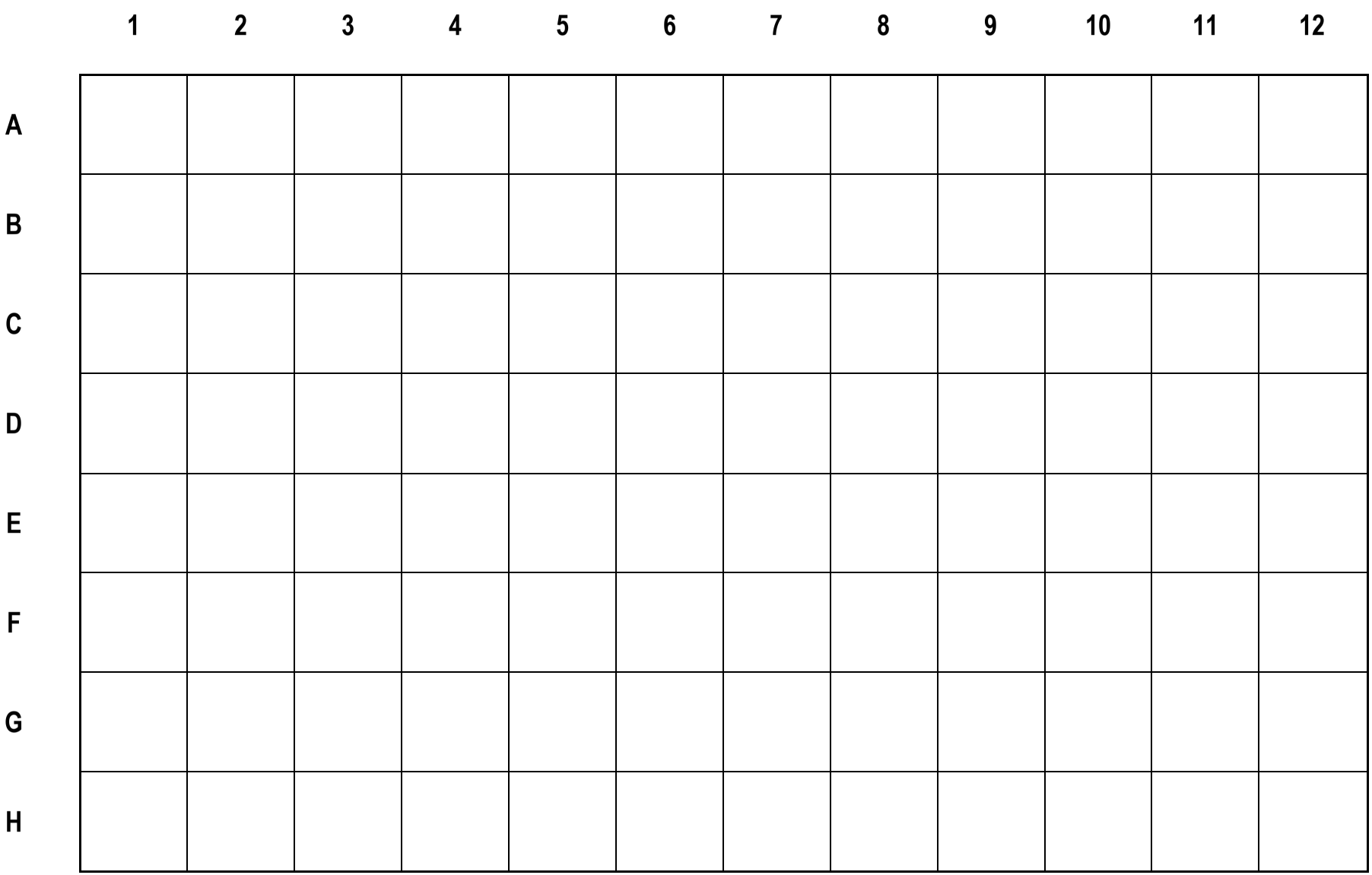
» **For more references on this product see our WebPages at www.biovendor.com**

19. EXPLANATION OF SYMBOLS

	Catalogue number
	Content
	Lot number
	Attention, see instructions for use
	Potential biological hazard
	Expiry date
	Storage conditions
	Name and registered office of the manufacturer

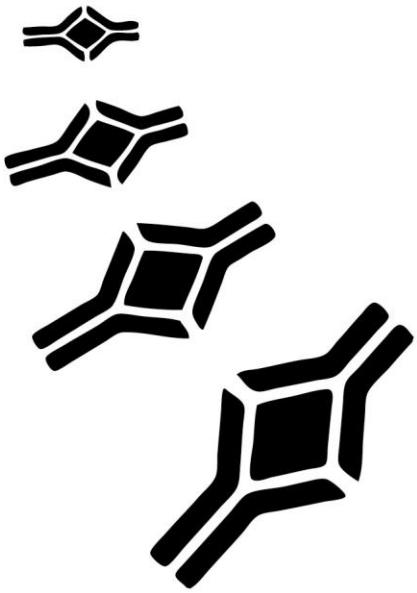
Assay Procedure Summary




1
2
3
4
5
6
7
8
9
10
11
12
A
B
C
D
E
F
G
H

NOTES

NOTES



BioVendor – Laboratorní medicína a.s.
Karasek 1767/1, 621 00 Brno, Czech Republic
Phone: +420-549-124-185, Fax: +420-549-211-460
E-mail: info@biovendor.com, sales@biovendor.com
Web: www.biovendor.com

There are BioVendor branches and distributors near you.
To find the office closest to you, visit **www.biovendor.com/contact**

BioVendor
Research
and Diagnostic Products