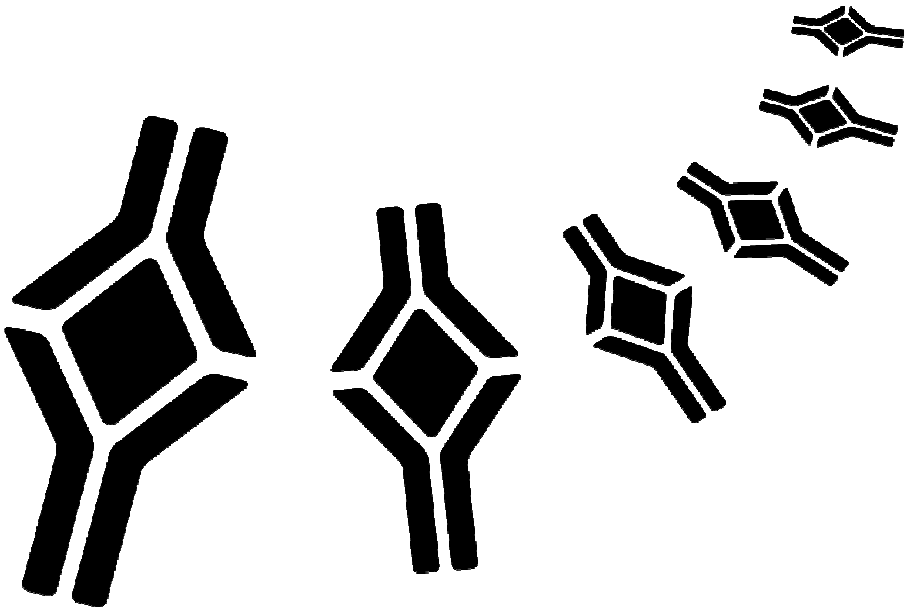
BioVendor
Research
and Diagnostic Products



HUMAN SUPAR ELISA

Product Data Sheet

Cat. No.: RD191408200R

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 RD191408200R Human suPAR ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human soluble urokinase-type plasminogen activator receptor (suPAR).

Features

- **It is intended for research use only**
- The total assay time is less than 3,5 hours
- The kit measures human suPAR in serum, plasma (EDTA, citrate, heparin) and urine
- Assay format is 96 wells
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2–8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

The urokinase-type plasminogen activator system consists of a protease, a receptor (uPAR) and inhibitors [1]. uPAR was initially characterized as a cofactor for plasminogen activation by its ligand urokinase-type plasminogen activator (uPA or urokinase) [2].
Structurally, uPAR is a glycosylphosphatidylinositol (GPI)-anchored membrane glycoprotein encoded by *PLAUR* gene. It consists of three homologous domains (DI, DII and DIII), each of approximately 90 amino acids. The molecular mass of non-glycosylated uPAR is approximately 35 kDa, whereas glycosylated uPAR has a molecular mass of approximately 60 kDa [2]. Removal of the GPI anchor by phospholipases or extracellular proteolytic cleavage yields a soluble form - soluble urokinase-type plasminogen activator receptor (suPAR) [3]. Cleavage of uPAR from the cell can occur both at the GPI-anchor and at the linker region between DI and DII [2]. Thus, suPAR is a circulating protein ranging from 20 to 50 kDa, depending on the degree of glycosylation and proteolytic cleavage [4].
The uPAR has been shown to associate with many signalling molecules and to mediate signal transduction. Chemotaxis-inducing molecules upregulate uPAR in different cell types, including neutrophils, macrophages, lymphocytes, endothelial cells and malignant cells. uPAR promotes the migration and adhesion of leucocytes by binding to β-integrins. Moreover, uPAR has a pivotal role in cell proliferation, angiogenesis and fibrinolysis [5, 6].
After cleavage from the cell surface, soluble uPAR can be measured in the blood and other organic fluids such as urine, saliva, bronchoalveolar lavage (BALF) and cerebrospinal fluid (CSF) [7]. In such matrices suPAR, similarly to anchored uPAR, also takes part in various immunological functions, including cell adhesion, migration, chemotaxis, proteolysis, immune activation, tissue remodelling, cell invasion and signal transduction [1].
Elevated levels of suPAR in circulation are considered to be a marker for activation of immune and inflammatory systems. In the acute setting, elevated levels of suPAR have been proposed to be predictive for disease severity in bacteraemia, human immunodeficiency virus infection (HIV), bacterial meningitis, active pulmonary tuberculosis, ventilator-associated pneumonia with sepsis, and in intensive care unit (ICU) patients with or without sepsis. The suPAR was also discovered as a cause of chronic renal disease focal segmental glomerulosclerosis (FSGS) [3, 6].
Described in details, suPAR levels were investigated as a predictor of disease severity and mortality in 132 patients with bacteremia caused by *Staphylococcus aureus*, *Streptococcus pneumonia* and *β-hemolytic streptococcae* or *Escherichia coli*. The best mortality predictive cut-off plasma level was 11 ng/ml; the sensitivity and specificity of suPAR for fatal disease was 83% and 76%, respectively [1, 5].
It was found that HIV-infected patients receiving antiretroviral therapy have an increased risk of various metabolic disorders, which may involve low-grade inflammation and other immunological perturbations. Plasma suPAR, which has been established as a marker of the immunological status of HIV-infected patients, may correlate with important features of dysmetabolism in such patients [8].
From the study performed with CSF samples of 545 patients was found that suPAR, age and

type of infection, all add value in predicting mortality among patients with initial diagnosis of meningitis [9].
The diagnostic value of suPAR to identify patients with severe sepsis seems to be similar to that of procalcitonin or interleukin-6. More importantly, suPAR levels was found to be strong predictors of 28-day, 90-day and even 1-year case fatality and allowed a better risk stratification compared to classical inflammatory markers such as procalcitonin, interleukin-6 or C-reactive protein [6, 7].
It has recently been suggested that suPAR has a role in the pathogenesis of focal segmental glomerulosclerosis (FSGS). *In vitro* and *in vivo* studies demonstrated that enhanced circulating suPAR deposits into the glomeruli, allowing activation of podocyte *β3*-integrin. This activation is sufficient to drive podocyte foot process effacement, proteinuria and initiation of FSGS [4, 10].
Lastly, plasma suPAR level was also shown to predict incident cancer [11, 12], cardiovascular disease [13], type 2 diabetes [14, 15] and mortality in the general population, suggesting that it might have a role as a biomarker of low-grade inflammation.

Areas of investigation:
Immune Response, Infection And Inflammation
- inflammation and low-grade inflammation
- infection diseases, sepsis and SIRS

Renal Disease

4. TEST PRINCIPLE

In the BioVendor Human suPAR ELISA, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-human uPAR(suPAR) antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-human uPAR(suPAR) antibody is added and incubated with the captured suPAR 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 suPAR. A standard curve is constructed by plotting absorbance values against suPAR 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	lyophilized	2 vials
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Dilution Buffer	ready to use	50 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 desiccant 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 suPAR 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 human suPAR in the stock solution is **500 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	–	500 pg/ml
250 µl of stock	250 µl	250 pg/ml
250 µl of 250 pg/ml	250 µl	125 pg/ml
250 µl of 125 pg/ml	250 µl	62.5 pg/ml
250 µl of 62.5 pg/ml	250 µl	31.3 pg/ml
250 µl of 31.3 pg/ml	250 µl	15.6 pg/ml
250 µl of 15.6 pg/ml	250 µl	7.8 pg/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 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 Biotin Labelled Antibody Concentrate 100x with Dilution Buffer (e.g. 10 µl of Biotin Labelled Antibody Concentrate + 990 µl of Dilution Buffer 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 the 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 suPAR in serum, plasma (EDTA, citrate, heparin) and urine.

Samples can be assayed immediately after collection, or after a long-term storage. 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 for serum and plasma is 15x.
Dilute samples (serum, plasma) 15x with the Dilution Buffer just prior to the assay as follows:
Add 20 µl of sample into 280 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Recommended starting dilution for urine is 20x.
Dilute urine samples urine 20x with the Dilution Buffer just prior to the assay as follows:
Add 15 µl of sample into 285 µl of Dilution Buffer. **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 suPAR.

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 suPAR 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 500**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
B	**Standard 250**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
C	**Standard 125**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
D	**Standard 62.5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
E	**Standard 31.3**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
F	**Standard 15.6**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
G	**Standard 7.8**	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 suPAR (pg/ml) in samples.

Alternatively, the logit log function can be used to linearize the standard curve, i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of Standards.

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because they have been diluted prior to the assay, e.g. 125 pg/ml (from standard curve) x 15 (dilution factor) = 1875 pg/ml.

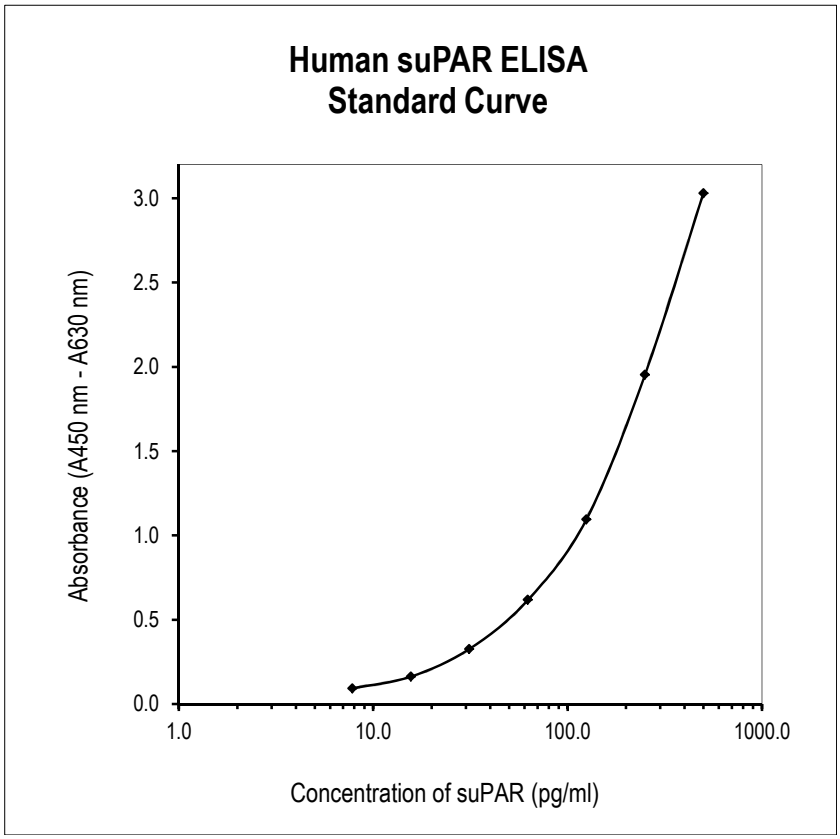


Figure 2: Typical standard curve for Human suPAR ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human suPAR 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 human suPAR values in wells and is 5.1 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 (pg/ml)*	*SD (pg/ml)*	*CV (%)*
Serum 1	2101.8	108.4	5.2
Serum 2	969.1	51.3	5.3

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

Sample	*Mean (pg/ml)*	*SD (pg/ml)*	*CV (%)*
Serum 1	2143.1	116.6	5.4
Serum 2	1185.9	75.5	6.4

- **Spiking Recovery**

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

Sample	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
Serum 1	1645.5	-	-
	2336.8	2348.8	99.5
	3101.3	3051.7	101.6
	4480.5	4458.0	100.5
Serum 2	946.2	-	-
	1613.4	1649.5	97.8
	2254.8	2352.4	95.8
	3577.2	3758.7	95.2

Sample	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
Urine 1	462.0	-	-
	745.7	774.5	96.3
	997.6	1087.0	91.8
	1533.2	1712.0	89.6
Urine 2	1113.9	-	-
	1687.1	1738.9	97.0
	2323.9	2363.9	98.3
	3372.5	3613.9	93.3

- **Linearity**

Serum and urine samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
Serum 1	-	2280.1	-	-
	2x	1130.7	1140.1	99.2
	4x	604.9	570.0	106.1
	8x	319.7	285.0	112.2
Serum 2	-	1629.8	-	-
	2x	822.9	814.9	101.0
	4x	407.4	407.5	100.0
	8x	220.2	203.7	108.1

Sample	*Dilution*	***O**bserved (pg/ml)*	***E**xpected (pg/ml)*	*Recovery **O/E** (%)*
Urine 1	-	7824.2	-	-
	2x	4012.0	3912.1	102.6
	4x	1972.6	1956.0	100.8
	8x	1004.8	978.0	102.7
Urine 2	-	5084.0	-	-
	2x	2587.6	2542.0	101.8
	4x	1273.4	1271.0	100.2
	8x	649.9	635.5	102.3

- **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 (pg/ml)*	*Plasma (pg/ml)*		
		EDTA	*Citrate*	*Heparin*
1	1751.5	1599.3	1201.0	1906.8
2	1883.5	1477.7	1266.6	1714.8
3	1037.4	944.2	677.0	979.9
4	1179.9	982.4	778.6	865.5
5	1382.5	1072.0	980.7	1115.2
6	1158.0	934.8	809.4	971.3
7	1151.0	844.6	685.2	922.0
8	1565.1	1477.8	1536.7	1241.8
9	1258.6	1137.9	891.6	1069.2
10	1213.4	1189.6	970.5	1112.6
Mean (pg/ml)	**1358.1**	**1166.0**	**979.7**	**1189.9**
Mean Plasma/Serum (%)		**85.9%**	**72.1%**	**87.6%**
Coefficient of determination R^2		**0.82**	**0.69**	**0.84**

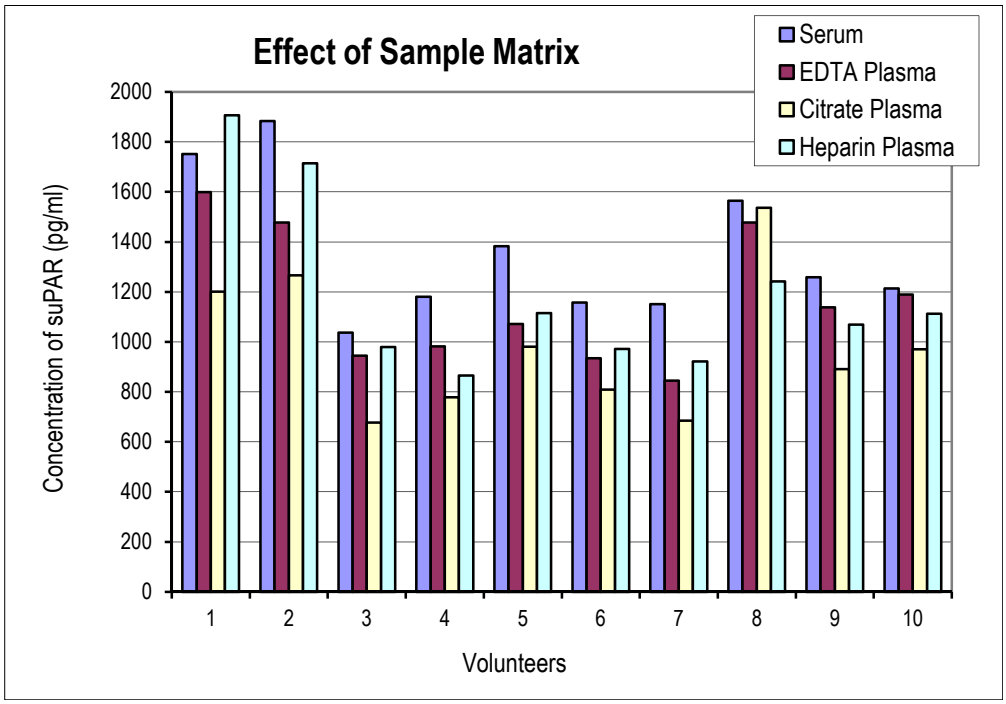


Figure 3: suPAR levels measured using Human suPAR ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

14. DEFINITION OF THE STANDARD

In this assay the recombinant protein (NS0 cell line from murine myeloma) is used as the standard. The recombinant suPAR protein is a 31 kDa protein consisting of 281 amino acid residues of the human uPAR with a C-terminal 6-His tag.

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 suPAR ELISA in our laboratory.

Sex	*Age (years)*	*n*	*suPAR (pg/ml)*				
			Mean	*Median*	*SD*	*Min*	*Max*
Men	20-29	18	1389.6	1381.9	233.2	854.4	1839.9
	30-39	26	1571.9	1495.2	379.4	893.3	2553.5
	40-49	31	1697.6	1604.6	367.9	1208.6	2614.5
	50-65	14	1761.3	1710.1	347.3	1161.4	2551.9
Women	20-29	12	1441.1	1389.7	250.6	1088.1	1904.5
	30-39	26	1562.5	1531.3	422.7	634.9	2591.0
	40-49	20	1643.9	1568.8	448.0	1076.9	2890.1
	50-61	8	1558.6	1591.1	321.9	1101.1	2038.8

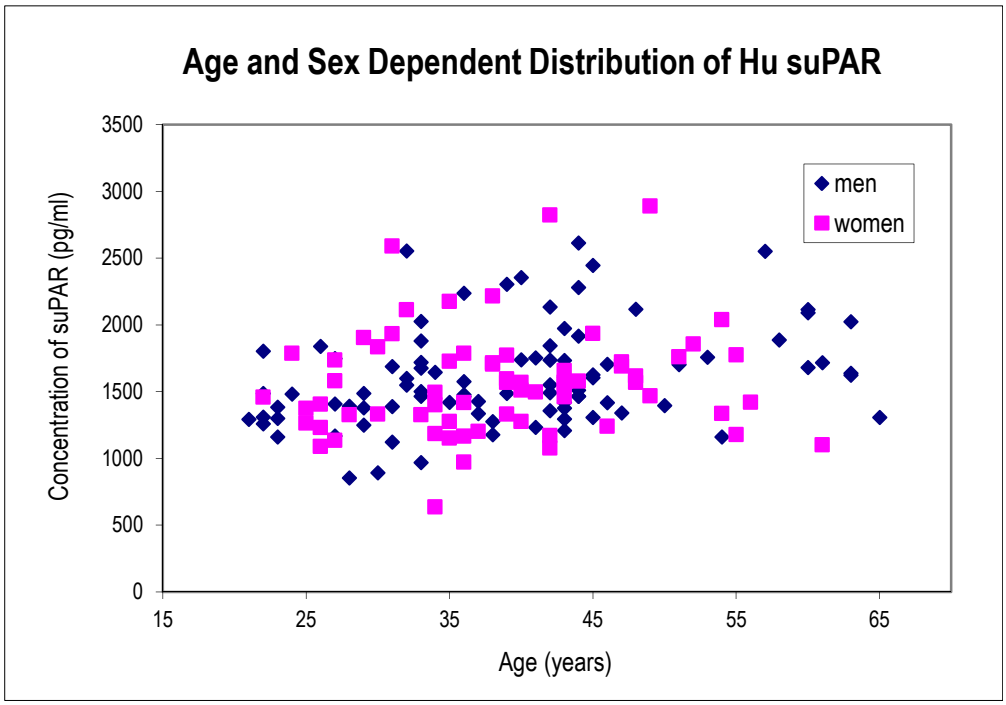


Figure 4: Human suPAR concentration plotted against donor age and sex.

- **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 suPAR levels with the assay.

16. METHOD COMPARISON

The BioVendor Human suPAR ELISA has been compared to another commercial immunoassay, by measuring 70 serum samples. Linear regression analysis of concentration data yielded the following results:

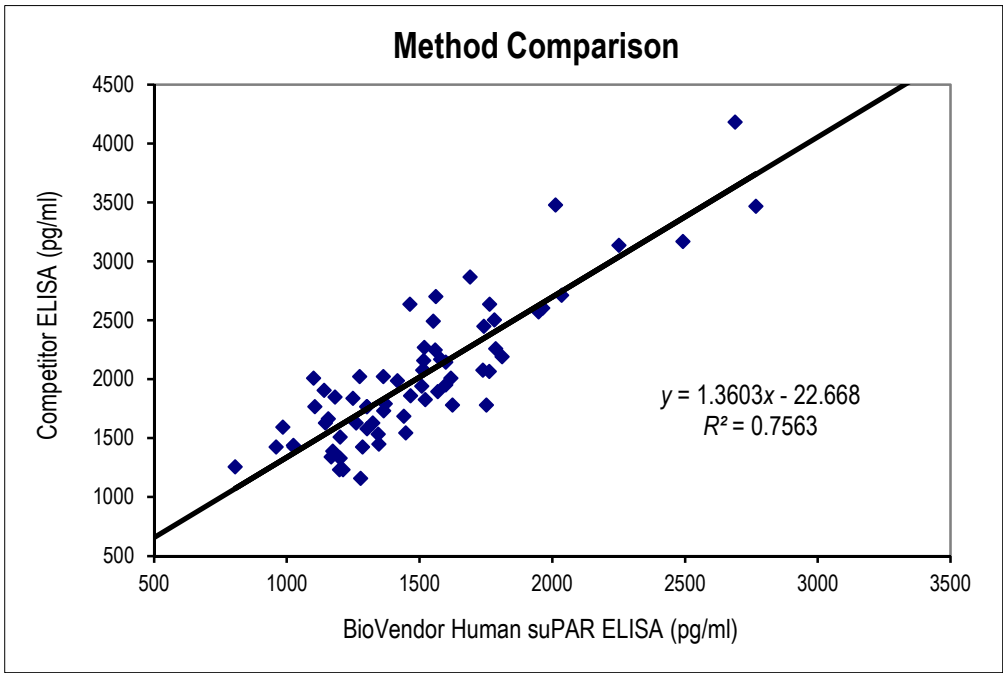


Figure 5: Method comparison.

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
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing standards or samples

18. REFERENCES

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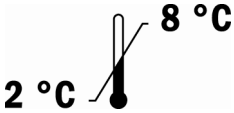
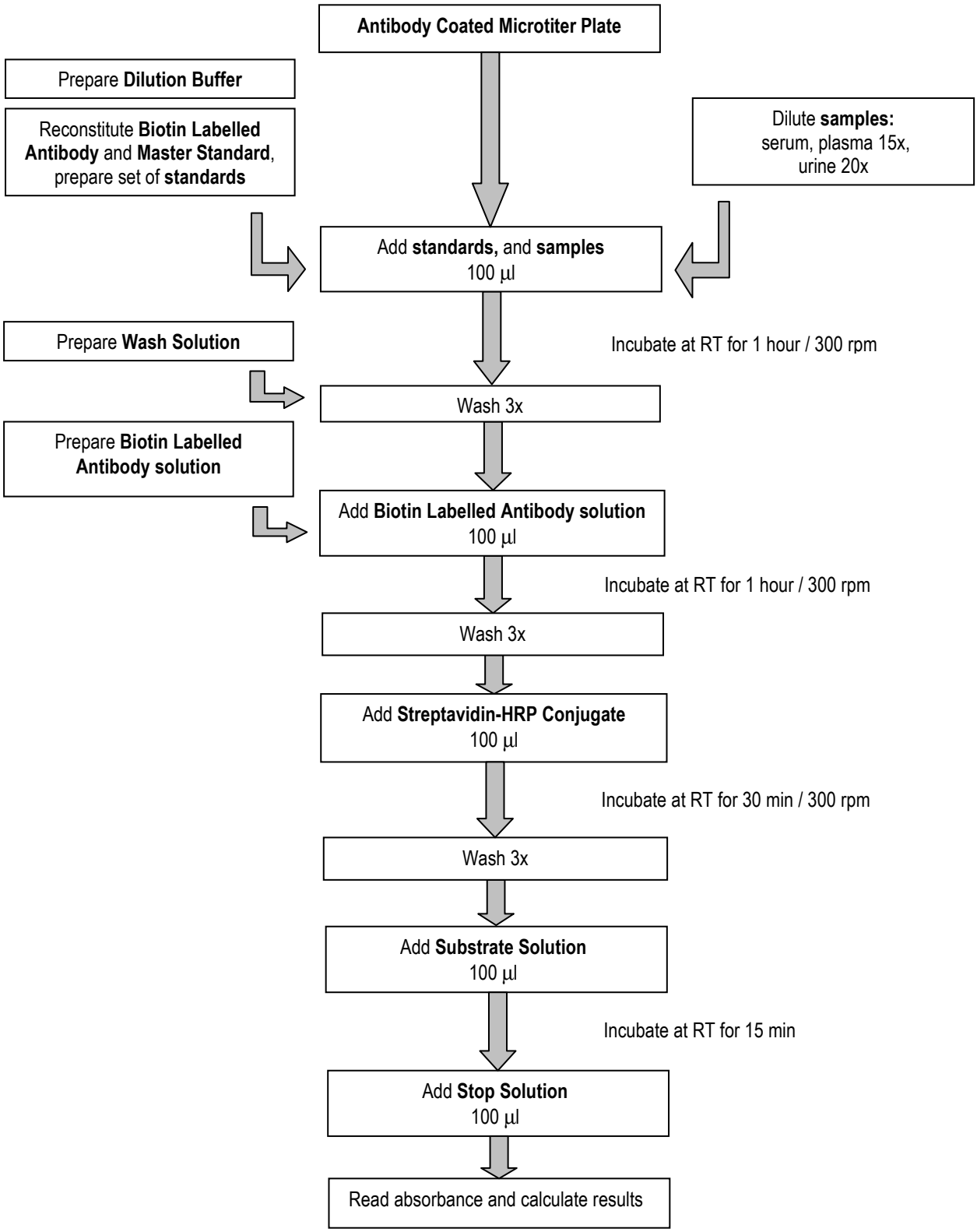
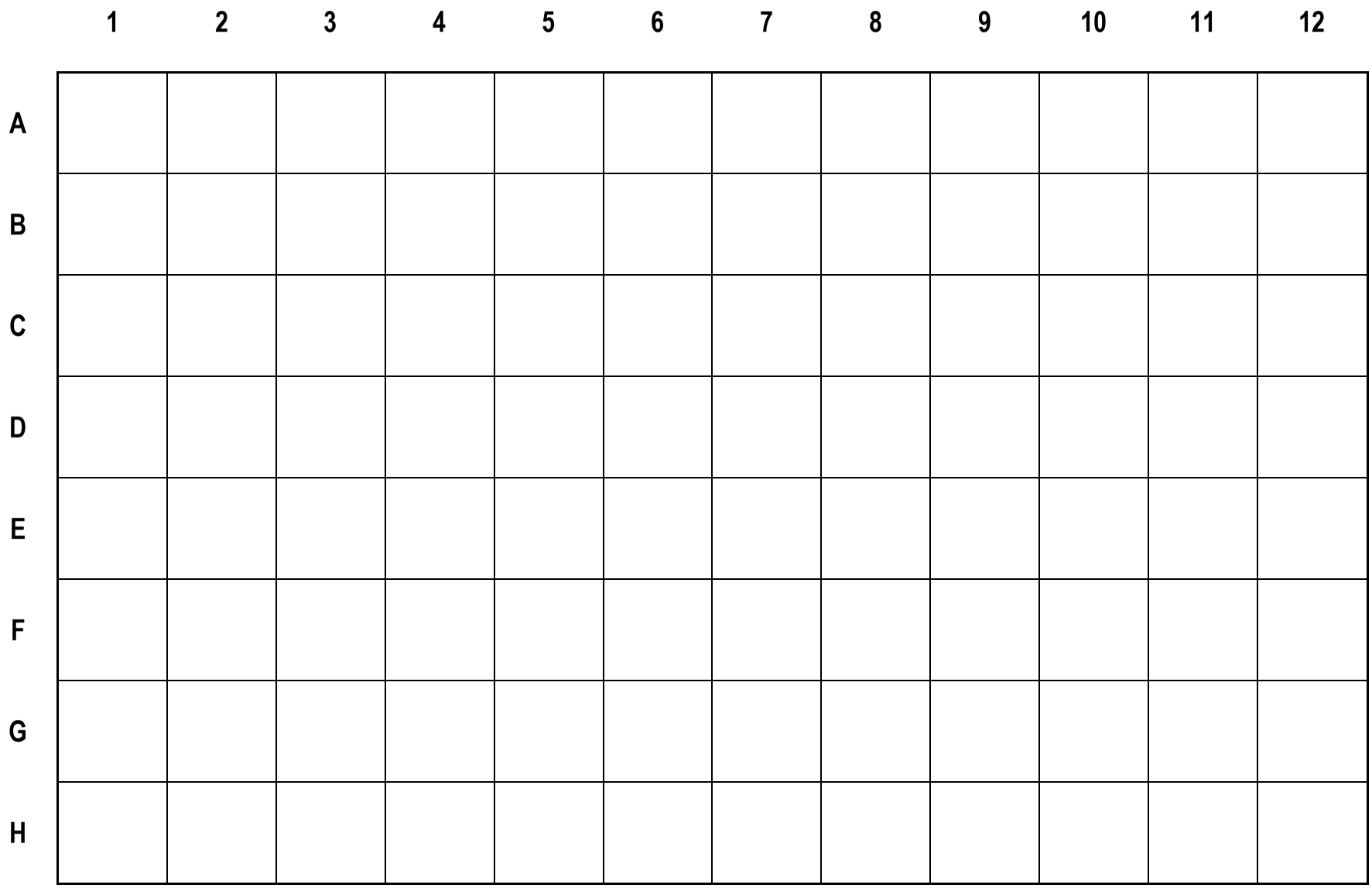
» For more references on this product see our WebPages at www.biovendor.com

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

Assay Procedure Summary

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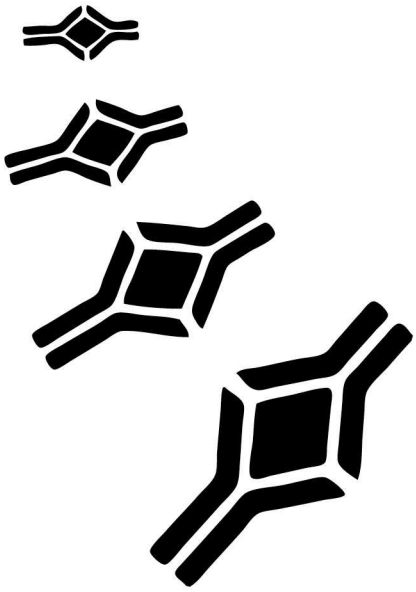

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