BioVendor Group

ENG

Product Data Sheet:

HUMAN SURFACTANT PROTEIN D ELISA

Catalogue number:

RD194059101

European Union:

IVD CE

Rest of the world:

For research use only!

BioVendor R&D

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CONTENTS

1. INTENDED USE

The RD194059101 Human Surfactant Protein D ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human surfactant protein D.

Features

- **European Union: for in vitro diagnostic use**
- **Rest of the world: for research use only!**
- The total assay time is less than 5 hours
- The kit measures total surfactant protein D in serum, plasma (EDTA, citrate, heparin), bronchoalveolar lavage fluid and amniotic fluid
- Assay format is 96 wells
- Quality Controls are human serum based. No animal sera are used
- 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

Human Surfactant Protein D (SP-D) is a member of the collagenous subfamily of glycoproteins and calcium-dependent lectins (collectins).

SP-D is a homotrimeric protein consisting of three 43kDa units that are bonded at their N-termini. Most preparations of SP-D contain predominantly dodecamers (four trimeric subunits), but also higher multimers have been observed. Each unit consists of at least four discrete structural domains: a short N-terminal domain; a relatively long collagenous domain, a short amphipathic connecting peptide, and a C-terminal, C- type lectin carbohydrate recognition domain (CRD).

SP-D is synthesized and secreted by two types of non-ciliated epithelial cells in the peripheral airway, alveolar type II cells and Clara cells. It is also expressed by various epithelial cells in the gastrointestinal and genitourinary tracts and placenta.

In the lungs, SP-D participates in the innate response to inhaled microorganisms and organic antigens. SP-D acts by aggregating bacteria and viruses, leukocyte function and stimulating an allergenic response. SP-D binds to the surface glycoconjugates of various microorganisms (eg, influenza virus, HIV, HSV, RSV, Mycoplasma pneumoniae) and the oligosaccharides associated with the surface of numerous organic antigens and enhances their phagocytosis. Studies have shown that SP-D binds to T cells, thus inhibiting their proliferation. SP-D also binds with inflammatory ligands via protein-protein and protein-carbohydrate interactions that are effective in reducing specific inflammation. In addition, SP-D binds to apoptotic cells and stimulates their phagocytosis by macrophages governed by mechanisms dependent and CD91 calreticulin.

Given that SP-D together with SP-A affects the reactivity of immune cells, their presence in the endometrium and placenta plays an important role in protection against bacteria and toxins during pregnancy. Reduced levels of all components of pulmonary surfactant, including SP-D, has been linked to premature birth.

Disturbance of pulmonary surfactant is in many cases the reason for collapse of the lungs and is also associated with many pulmonary diseases. All types of chronic lung disease is characterized by pathologically altered levels in lung tissue (fibrosis and emphysema). Studies have shown that expression of SP-D is associated with many pulmonary diseases: cystic fibrosis, acute interstitial pneumonia (ARDS), chronic obstructive pulmonary disease, asthma, bronchopulmonary dysplasia, alveolar capillary dysplasia, alveolar proteinase and tuberculosis.

<u>Clinical application and areas of investigation:</u>

Cystic fibrosis

Acute interstitial pneumonia (ARDS)

Chronic obstructive pulmonary disease

Asthma

Bronchopulmonary dysplasia

Alveolar capillary dysplasia and alveolar proteinase

Immune response, infection and inflammation

4. TEST PRINCIPLE

In the BioVendor Human Surfactant Protein D ELISA, Standards, Quality Controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human surfactant protein D antibody. After 120 minutes incubation and washing, biotin labelled monoclonal anti-human SP-D antibody is added and incubated with the captured SP-D for 60 minutes. After another washing, Streptavidin-HRP Conjugate is added. After 60 minutes incubation and the last 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 surfactant protein D. 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. However, 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
Biotin Labelled Antibody	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	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

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 microtiter 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 desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months stored at 2-8°C and protected from the moisture.

Biotin Labelled Antibody

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:

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 SP-D 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
300 µl of stock	300 µl	50 ng/ml
300 µl of 50 ng/ml	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

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

Stability and storage:

Reconstituted Master Standard (100 ng/ml) must be used immediately. 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!!!

The volume of dilution buffer for reconstitution of Quality Controls given in CoA dilutes Quality Controls 11x, the same as samples.

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).

Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:

Do not store the reconstituted Quality Controls.

Note:

Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

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 Surfactant Protein D in serum and plasma (EDTA, citrate, heparin), bronchoalveolar lavage fluid and amniotic fluid.

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 11x with Dilution Buffer just prior to the assay, e.g. 25 µl of sample + 250 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.

In case of measurement of SP-D in bronchoalveolar lavage fluid (BAL) an appropriate dilution should be assessed by the researcher in advance to batch measurement (recommended starting dilution is 40x, e.g. 10 µl of BAL sample + 390 µl of Dilution Buffer).

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 Surfactant Protein D.

Note: It is recommended to use a precision pipette and a careful technique to perform the dilution in order to get precise results.

Ask for protocol at info@biovendor.com if assaying bronchoalveolar lavage fluid and amniotic fluid.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of 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 23-27°C for **2 hours**, 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. For manual washing, see *Note 2*!
4. Add **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at 23-27°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. For manual washing, see *Note 2*!
7. Add **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at 23-27°C for **1 hour**, 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. For manual washing, see *Note 2*!
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 23-27°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 SP-D 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: Remove liquid from wells completely and pipet 0.35 ml Wash Solution into each well. Remove solution from wells completely and repeat washing four times. After final wash, invert and tap the plate strongly against paper towel. **Make certain that liquid has been removed from wells entirely after each washing step.**

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 100**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 50**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 25**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 12.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 6.25**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 3.13**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 1.56**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
H	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38

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 Surfactant Protein D (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 and Quality Controls calculated from the standard curve must be multiplied by their respective dilution factor, because samples and controls have been diluted prior to the assay, e.g. 13.5 ng/ml (from standard curve) x 11 (dilution factor) = 148.5 ng/ml.

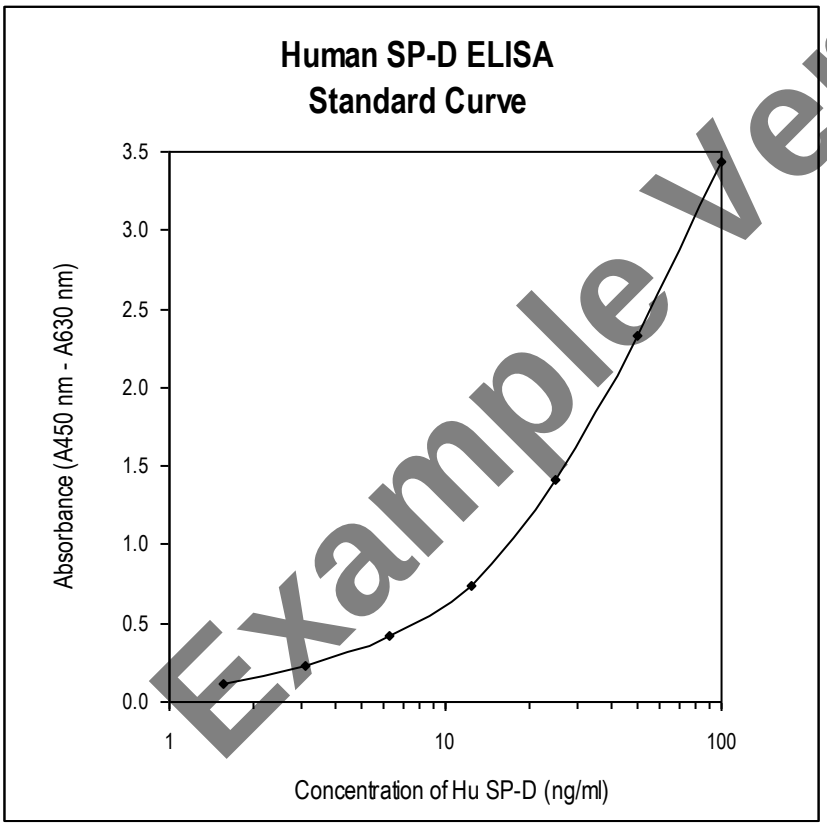

Figure 2: Typical Standard Curve for Human SP-D ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Surfactant Protein D 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 SP-D values in wells and is 0.1 ng/ml.

*Dilution Buffer is pipetted into blank wells.

Limit of assay

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

Specificity

The monoclonal antibodies used in this ELISA are specific for human surfactant protein D.

Determination of surfactant protein D does not interfere with hemoglobin (2.0 mg/ml), bilirubin (340 µmol/l), triglycerides 22.6 mmol/l) and biotin (3500 ng/ml).

Sera of several mammalian species were measured in the assay. See results below.

For details please contact us at info@biovendor.com.

Mammalian serum	Observed
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 (ng/ml)	SD (ng/ml)	CV (%)
1	214.05	16.44	7.7
2	599.09	31.00	5.2

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	174.89	10.18	5.8
2	286.04	8.71	3.0

Spiking Recovery

Serum samples were spiked with different amounts of human SP-D and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	64.20	-	-
	196.47	201.70	97.4
	125.29	132.95	94.2
	98.43	98.57	99.9
Serum 2	111.38	-	-
	341.18	386.38	88.3
	243.06	248.88	97.7
	188.49	180.13	104.6
BAL	175.96	-	-
	476.33	450.96	105.6
	341.21	313.46	108.9
	291.97	244.71	119.3
Amniotic fluid	31.54	-	-
	93.58	100.29	93.3
	63.62	65.91	96.5
	54.77	48.73	112.4

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	-	216.07	-	-
	2x	102.65	108.04	95.0
	4x	55.52	54.02	102.8
	8x	29.30	27.01	108.5
Serum 2	-	280.23	-	-
	2x	137.59	140.11	98.2
	4x	79.26	70.06	113.1
	8x	38.68	35.03	110.4
BAL	-	461.81	-	-
	2x	232.28	230.91	100.6
	4x	118.60	115.45	102.7
	8x	59.11	57.73	102.4
Amniotic fluid	-	8160.00	-	-
	2x	4327.52	4080.00	106.1
	4x	1948.24	2040.00	95.5
	8x	1113.20	1020.00	109.1

Effect of sample matrix

Heparin, citrate and EDTA 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	47.43	42.44	43.44	52.43
2	72.52	57.54	58.36	71.50
3	140.04	122.74	107.35	154.04
4	83.24	71.96	72.25	62.38
5	137.07	126.36	124.06	144.39
6	158.47	125.17	127.25	159.39
7	72.95	74.40	75.49	92.00
8	123.57	116.55	110.36	143.77
9	78.20	59.94	61.83	80.61
10	151.66	130.66	128.55	151.95
Mean (ng/ml)	**106.52**	**92.78**	**90.89**	**111.25**
Mean Plasma/Serum (%)		**87.1**	**85.3**	**104.4**
Coefficient of determination R^2		**0.95**	**0.95**	**0.92**

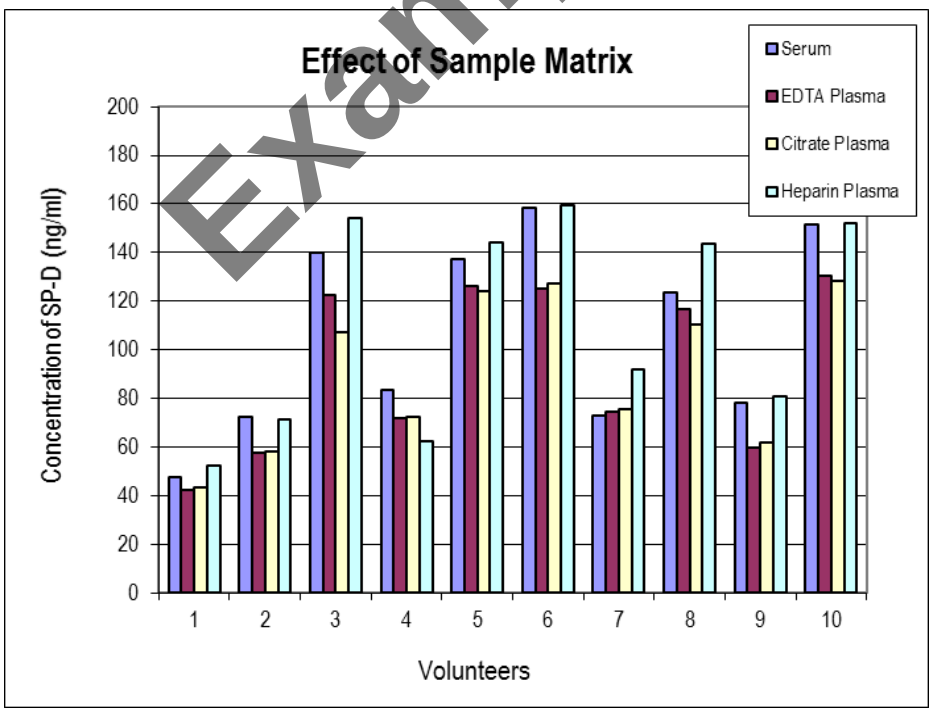

Figure 3: SP-D levels measured using Human SP-D ELISA from 10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

Stability of samples stored at 2-8°C

Samples should be stored at -20°C, or preferably at -70°C. However, no decline in concentration of SP-D was observed in serum and plasma samples after 7 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 (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	-20°C	105.18	92.41	86.32	93.13
	2-8°C, 1 day	89.66	85.38	83.61	93.31
	2-8°C, 7 days	90.72	76.62	78.70	88.71
2	-20°C	205.30	165.84	175.85	161.44
	2-8°C, 1 day	206.10	162.90	171.28	162.61
	2-8°C, 7 days	187.85	157.67	167.62	146.49
3	-20°C	64.72	52.51	55.14	61.84
	2-8°C, 1 day	65.36	51.75	50.46	58.69
	2-8°C, 7 days	58.98	47.71	52.61	58.86

Effect of Freezing/Thawing

No decline was observed in concentration of human SP-D 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 (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	1x	91.29	92.79	83.35	70.95
	3x	95.32	91.29	83.95	74.84
	5x	94.26	97.79	83.63	73.78
2	1x	57.24	50.27	48.13	52.63
	3x	52.50	50.14	49.59	52.50
	5x	54.54	48.67	51.70	52.89
3	1x	44.02	31.27	31.98	48.48
	3x	40.40	23.79	24.02	40.09
	5x	38.43	22.90	30.11	40.62

14. DEFINITION OF THE STANDARD

A recombinant protein is used as the standard. The recombinant human SP-D is oligomer consisting of 355 amino acid polypeptide chains.

15. PRELIMINARY POPULATION AND CLINICAL 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 Sufactant Protein D ELISA kit in our laboratory.

The presented data should be regarded only as guideline.

Age dependent distribution of SPD

Sex	Age (years)	n	SP-D (ng/ml)				
			Mean	Median	SD	Min	Max
Men	20-29	17	126.1	125.1	45.6	39.8	234.6
	30-39	25	139.6	128.8	77.5	28.5	324.2
	40-49	31	129.5	106.1	52.3	46.3	279.0
	50-65	16	120.0	99.1	71.0	52.7	340.4
Women	20-29	12	115.9	115.4	36.2	57.0	211.5
	30-39	26	109.7	89.0	80.0	52.8	450.3
	40-49	20	120.4	104.6	60.8	53.5	258.5
	50-61	8	126.3	114.9	33.5	81.9	191.2

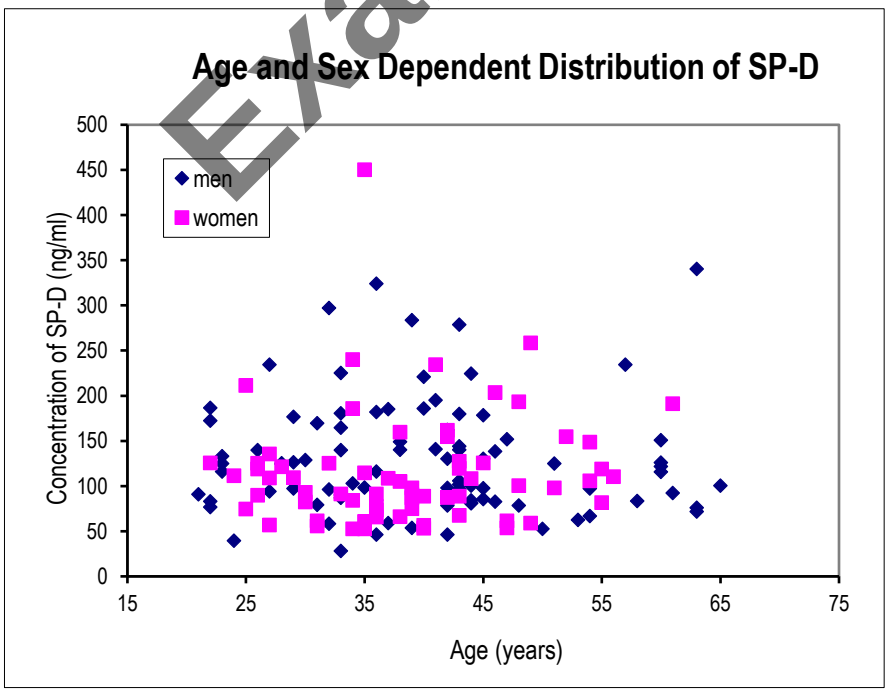

Figure 4: Human SPD concentration plotted against donor age and sex.

Distribution of SP-D in BALF and amniotic fluid samples

BALF samples were taken from 14 patients, amniotic fluid samples were taken from 10 women and measured in the assay. Results are shown below:

Samples	Mean (ng/ml)	Minimum value (ng/ml)	Maximum value (ng/ml)
BALF	363.24	14.98	2690.67
Amniotic fluid (first trimester)	21.05	9.63	38.71
Amniotic fluid (third trimester)	5561.55	3364.69	7875.27

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 SPD levels with the assay.

16. METHOD COMPARISON

The BioVendor Surfactant Protein D ELISA was compared to the previous version of the ELISA. The following correlation graph was obtained.

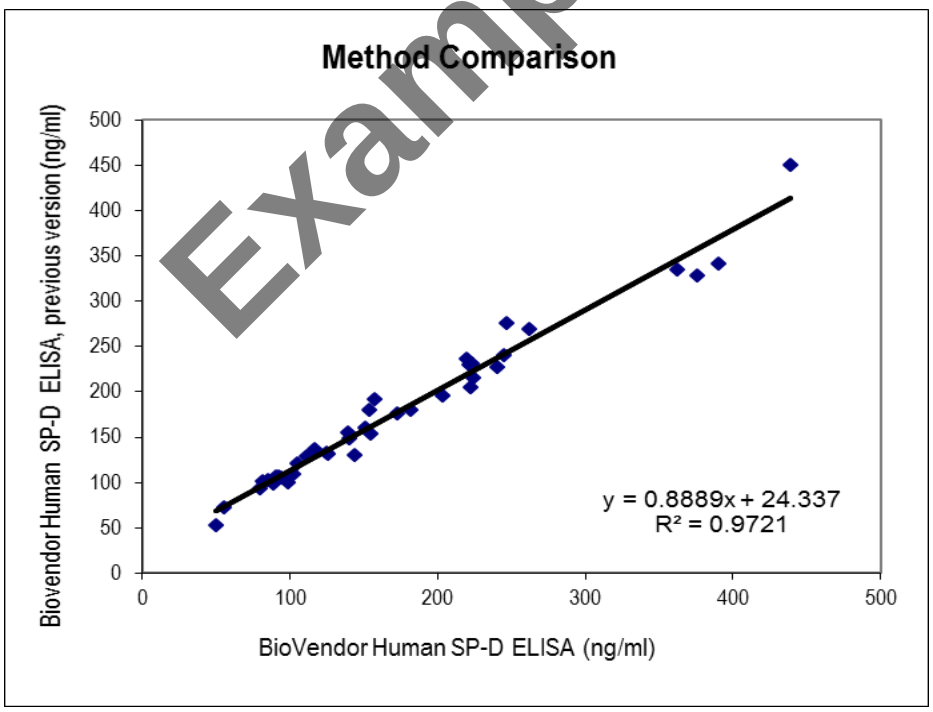

Figure 5: Method comparison.

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
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:

- Improper or inadequate washing
- Improper mixing Standards, Quality Controls or samples

High signal of controls and samples

Possible explanations:

- Incubation temperature over 30°C. Performing the incubation at the temperature of 23-27°C is crucial in order to obtain valuable results.

18. REFERENCES

References to SP-D:

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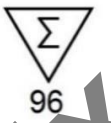
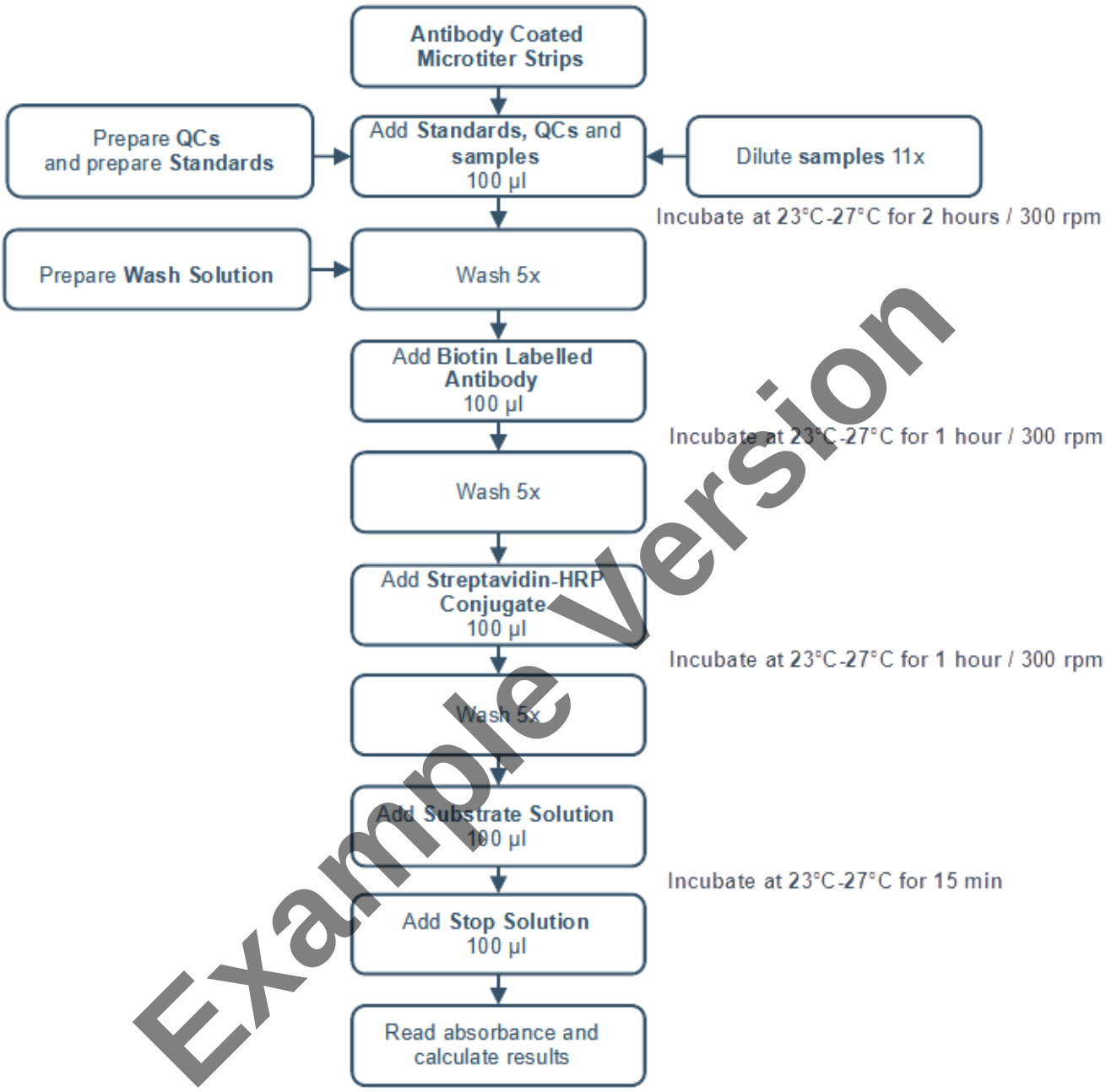
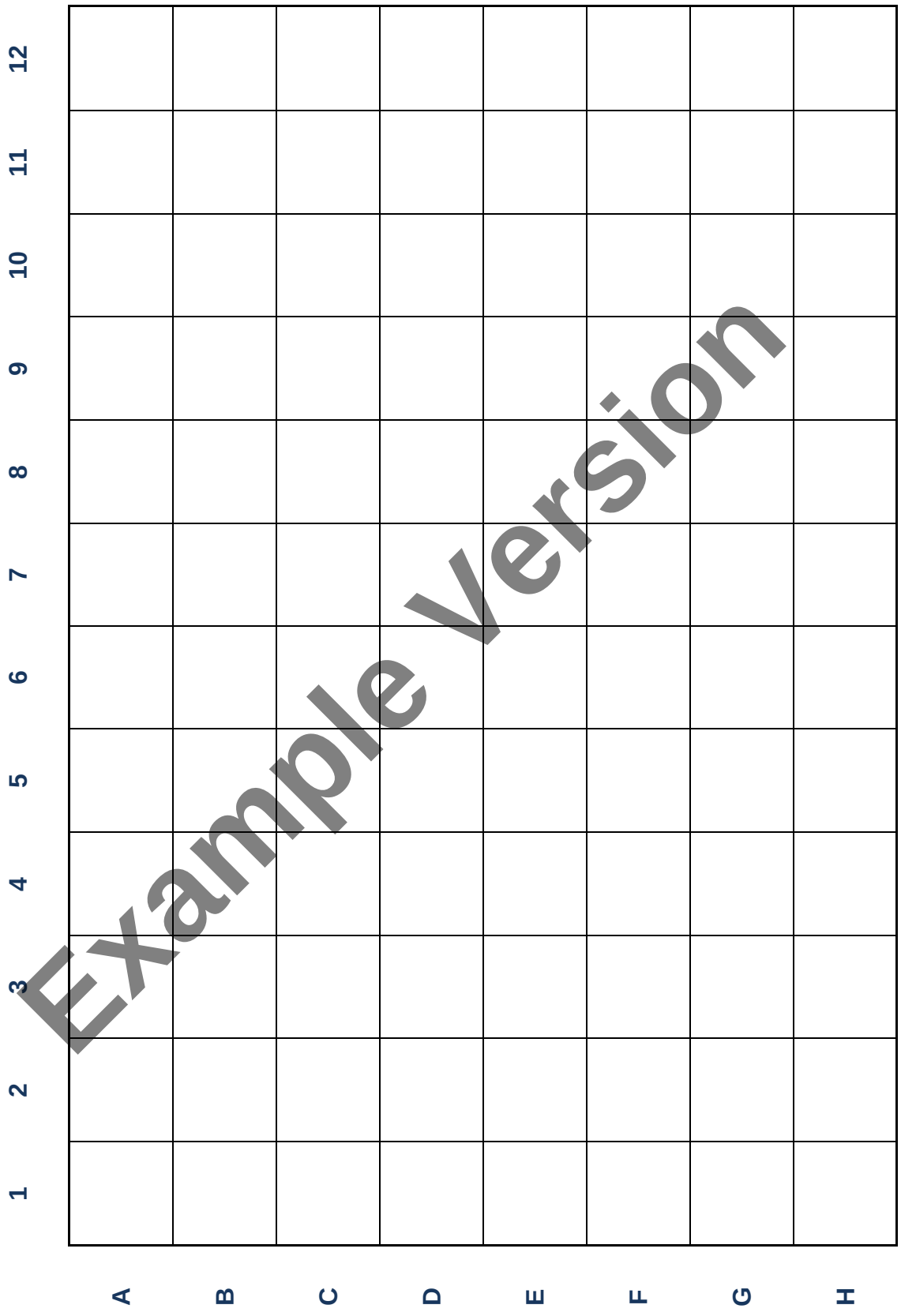
19. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks
	*In vitro* diagnostic medical device
	CE marking of conformity

20. ASSAY PROCEDURE - SUMMARY





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