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Research
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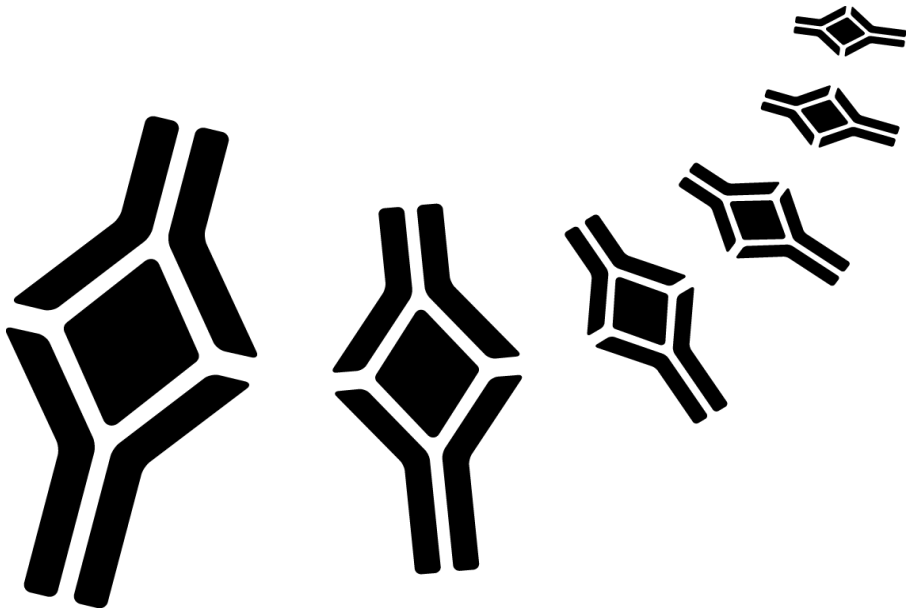

HUMAN S100A8/A9 (CALPROTECTIN) ELISA

Product Data Sheet

Cat. No.: RD191217100R

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 RD191217100R Human S100A8/A9 (Calprotectin) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human S100A8/A9 (calprotectin).

Features

- **It is intended for research use only**
- The total assay time is less than 2.5 hours
- The kit measures S100A8/A9 in human serum, plasma (EDTA, citrate, heparin) bronchoalveolar lavage fluid (BALF), cerebrospinal fluid (CSF), urine samples and stool samples
- Extraction Buffer (Cat. No.: C005821) needed for extraction of stool samples is not included and can be obtained separately. For details please contact us at info@biovendor.com
- Assay format is 96 wells
- Quality Controls are human serum based
- 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

S100A8/A9, also known as calprotectin or MRP8/14, is a heterocomplex of the two S100 calcium binding proteins, S100A8 (calgranulin A or MRP8 – myeloid related protein 8) and S100A9 (calgranulin B or MRP14 – myeloid related protein 14) [1]. S100A8 has a molecular weight of 11.0 kDa and S100A9 exists in two forms, 13.3 kDa and truncated 12.9 kDa. Both proteins are similar to other members of the S100 family in that they contain two EF-hand motifs that bind calcium ions. Ca^{2+}-binding induces the formation of heterocomplexes S100A8/S100A9 and $(S100A8)_2$ /$(S100A9)_2$ [2,3].
S100A8 and S100A9 are expressed in a tissue/cell-specific manner mainly in cells of the myeloid lineage, such as granulocytes, monocytes and early stages of macrophages, but not in resident tissue macrophages [2]. They are also expressed in keratinocytes and epithelial cells but only under inflammatory conditions. S100A8/A9 complex is an antimicrobial peptide that is released by innate immunity cells immediately after host pathogen interaction, protects cells against invasive microorganisms, and regulates adhesion of leucocytes to the endothelium and extracellular matrix during the inflammatory process [6].
S100A8/A9 has emerged as a very promising biomarker for a wide range of inflammatory processes such as rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, inflammatory bowel disease (IBD), acute lung inflammation and vasculitis [10,12,13,14,16]. Fecal S100A8/A9 level reflects the severity of mucosal inflammation and is a good diagnostic marker for monitoring of IBD (Crohn's disease, ulcerative colitis) and neoplasm [4,5]. S100A8/A9 serum levels have been identified as independent risk predictors for various cardiovascular diseases such as acute coronary syndrome and myocardial infarction [8]. High circulating levels of S100A8/A9 complex were measured in patients with abdominal adiposity and correlated with visceral fat area, body mass index, subcutaneous fat area, and leukocyte count [11]. S100A8 and S100A9 play a critical role in tumor biology and their elevated levels were found in numerous tumors. In cancer progression low concentrations of S100A8/A9 complexes promote tumor cell growth and tumor cell migration, while high concentrations are associated with apoptotic effects on tumor cells [9]. Measuring urinary calprotectin shows potential in the differential diagnosis of acute kidney injury (AKI) [15].

<u>Areas of investigation:</u>

Inflammatory bowel disease
Rheumatoid Arthritis
Obesity
Carcinomas
Cardiovascular diseases
Kidney injury

4. TEST PRINCIPLE

In the BioVendor Human S100A8/A9 ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human S100A9 antibody. After 60 minutes incubation and washing, HRP labelled polyclonal anti-human S100A8 antibody is added and incubated with captured S100A8/A9 for 60 minutes. After another 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 S100A8/A9. 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**
- **Notice: Wear gloves, face mask (or another mouth covering) and laboratory coat when handling ELISA components and during ELISA assay. Skin and saliva can contain S100A8/A9 protein and contamination in any ELISA step could cause false positive results**
- 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
Conjugate Solution Conc. (100x)	concentrated	0.13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Conjugate Diluent	ready to use	13 ml
Dilution Buffer	ready to use	100 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 550-650nm)
- 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 month stored at 2-8°C and protected from the moisture.

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

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

Human S100A8/A9 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 S100A8/A9 in the stock solution is **64 ng/ml**.
Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	64 ng/ml
250 µl of stock	250 µl	32 ng/ml
250 µl of 32 ng/ml	250 µl	16 ng/ml
250 µl of 16 ng/ml	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

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.

Quality Controls HIGH, LOW
Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
The reconstituted Quality Controls are ready to use, do not dilute them.
Stability and storage:
Do not store 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 the kit works in accordance with PDS and CoA and that ELISA test was carried out properly.

Conjugate Solution Conc. (100x)
Prepare the working Conjugate Solution by adding 1 part Conjugate Solution Concentrate (100x) to 99 parts Conjugate Diluent. Example: 10 µl of Conjugate Solution Concentrate (100x) + 990 µl of Conjugate Diluent for 1 strip (8 wells).
Stability and storage:
Opened Conjugate Solution Concentrate (100x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Conjugate 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 human S100A8/A9 in serum, plasma (EDTA, citrate, heparin), BALF, CSF, urine and stool samples.

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

Collection of blood samples must be performed carefully because human S100A8/A9 (calprotectin) can be released from neutrophils into surrounding fluid during the incorrect process of blood coagulation leading to elevated serum or plasma levels and false positive results.

Serum and plasma samples:
Dilute serum samples **200x** with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 995 µl of Dilution Buffer for singlets and duplicates. **Mix well** (not to foam). Vortex is recommended.

BALF samples:
Dilute BALF samples **100x** with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 495 µl of Dilution Buffer for singlets and duplicates. **Mix well** (not to foam). Vortex is recommended.

CSF samples:
Dilute CSF samples **3x** with Dilution Buffer just prior to the assay, e.g. 50 µl of sample + 100 µl of Dilution Buffer for singlets and 100 µl of sample + 200 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Urine samples:
Dilute urine samples just prior to the assay **25x** with Dilution Buffer, e.g. 6 µl of sample + 144 µl of Dilution Buffer for singlets or 10 µl of sample + 240 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Serum samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine, BALF and CSF samples should be stored at -70°C.
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 human S100A8/A9.

Stool samples:
Collection and extraction:
Collect 50 to 100 mg of stool for extraction procedure – add BioVendor Extraction Buffer (Cat. No.: C005821) to polypropylene tube with known weight of stool samples giving a dilution factor 50x, e.g. if stool weight is 55 mg add 2695 µl of Extraction Buffer [55 (weight) x 50 (dilution factor) – 55 (weight) = 2695 µl]. Homogenize the samples on a vortex at high speed for 30 minutes and centrifuge for 5 minutes at 3000 g. Use supernatant for analysis in ELISA.

Dilute stool extract **200x** with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 995 µl of Dilution Buffer for singlets and duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Stool samples should be stored at 2-8°C up to 6 days and for long-term storage should be stored at -20°C, or preferably at -70°C. Extract should be storage at -20°C, or preferably at -70°C for at least 3 months. 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 diluted standards, Quality Controls, Dilution Buffer (=Blank) and samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Add **100 µl** of Conjugate Solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Add **100 µl** of Substrate Solution into each well. Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.
8. Incubate the plate for **10 minutes** at room temperature. The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C. Do not shake the plate during the incubation.
9. Stop the colour development by adding **100 µl** of Stop Solution.
10. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 – 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 9.**

Note: 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 S100A8/A9 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 64**	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31
B	**Standard 32**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
C	**Standard 16**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
D	**Standard 8**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
E	**Standard 4**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
F	**Standard 2**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
G	**Standard 1**	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 S100A8/A9 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. 20 ng/ml (from standard curve) x 200 (dilution factor) = 4 000 ng/ml = 4 μg/ml.
For stool samples concentration must be multiplied by extraction dilution factor and respective ELISA dilution factor before assaying, e.g. 40 ng/ml (from standard curve) x 200 (ELISA dilution factor) x 50 (extraction dilution factor) = 400 000 ng/ml = 400 μg/ml = 400 μg/g of stool samples.

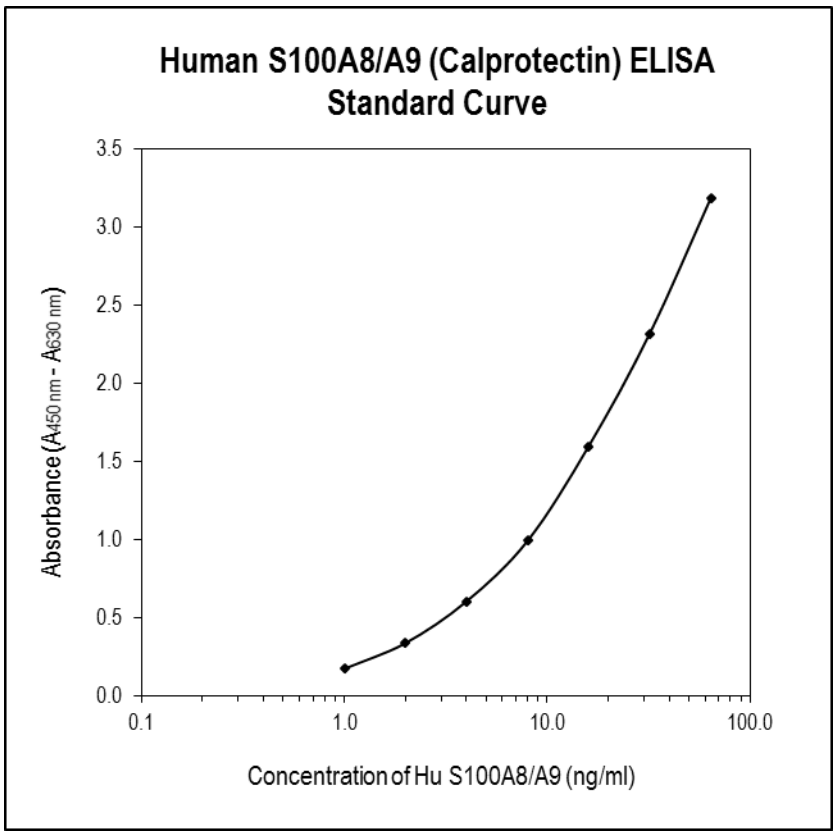

Figure 2: Typical Standard Curve for Human S100A8/A9 ELISA.

13. PERFORMANCE CHARACTERISTICS

» Typical analytical data of BioVendor Human S100A8/A9 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 S100A8/A9 values in wells and is 0.22 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.

- **Specificity**

The antibodies used in this ELISA are specific for human S100A8/A9. No crossreactivity has been observed for other human recombinant S100 proteins such as S100A1, A4, A5, A6, A7, A10, A11, A12, A13, A14, A15, A16, S100B and S100G protein at 200 ng/ml.
Determination of S100A8/A9 does not interfere with haemoglobin (0.05 mg/ml), bilirubin (170 μmol/l) and triglycerides (5.0 mmol/l).

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

Mammalian serum sample	*Observed crossreactivity*
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	no
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 (%)*
Serum 1	2338	69	3.0
Serum 2	4104	283	6.9

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
Serum 1	3296	134	4.1
Serum 2	4335	186	4.3

- **Spiking Recovery**

Samples were spiked with different amounts of calprotectin and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
Serum 1	700	-	-
	1065	1100	96.8
	1537	1500	102.5
	2402	2300	104.4
Serum 2	758	-	-
	1084	1158	93.6
	1599	1558	102.7
	2450	2358	103.9
CSF	14.7	-	-
	28.2	26.7	105.4
	37.0	38.7	95.5
	61.0	62.7	97.2
Urine	161	-	-
	231	261	88.7
	342	361	94.6
	487	561	86.7
BALF	419	-	-
	815	819	99.5
	1220	1219	100.1
	2253	2019	111.6

Sample	***O**bserved* (*µg/g*)	***E**xpected* (*µg/g*)	*Recovery **O/E** (%)*
Stool	34.7	-	-
	52.0	54.7	95.0
	69.0	74.7	92.4
	94.8	114.7	82.6

- **Linearity**

Samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved* (*ng/ml*)	***E**xpected* (*ng/ml*)	*Recovery **O/E** (%)*
Serum 1	-	3757	-	-
	2x	1881	1878	100.1
	4x	900	939	95.9
	8x	434	470	92.3
Serum 2	-	2690	-	-
	2x	1329	1347	98.7
	4x	656	673	97.5
	8x	233	337	96.1
CSF 1	-	117.8	-	-
	2x	65.6	58.9	111.4
	4x	34.8	29.5	118.3
	8x	17.2	14.7	117.1
CSF 2	-	44.7	-	-
	2x	19.1	22.4	85.6
	4x	10.1	11.2	90.3
	8x	5.1	5.6	92.0
Urine 1	-	674	-	-
	2x	332	337	98.6
	4x	156	169	92.4
	8x	75	84	88.6
Urine 2	-	825	-	-
	2x	425	412	103.0
	4x	216	206	104.8
	8x	100	103	97.1
BALF 1	-	1196	-	-
	2x	593	598	99.1
	4x	311	299	104.0
	8x	163	150	109.3
BALF 2	-	2831	-	-
	2x	1299	1416	91.8
	4x	729	708	103.0
	8x	318	354	89.9

Sample	*Dilution*	***O**bserved* (*µg/g*)	***E**xpected* (*µg/g*)	*Recovery* ***O/E** (%)*
Stool 1	-	471.6	-	-
	2x	279.8	235.8	118.7
	4x	131.5	117.9	111.6
	8x	62.2	59.0	105.5
Stool 2	-	556.7	-	-
	2x	328.5	278.4	118.0
	4x	150.8	139.4	108.4
	8x	70.2	69.6	100.9

- **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	2565	1890	1918	2183
2	1206	682	1135	1056
3	1189	466	906	919
4	956	387	753	931
5	2290	1391	2215	2464
6	1440	632	762	1307
7	1889	837	1106	1787
8	1252	790	1420	1063
9	1366	530	1161	1167
10	2875	1700	3185	3586
Mean (ng/ml)		**931**	**1456**	**1652**
Mean Plasma/Serum (%)	**-**	**54.6**	**85.5**	**97.0**
Coefficient of determination R^2	**-**	**0.90**	**0.78**	**0.91**

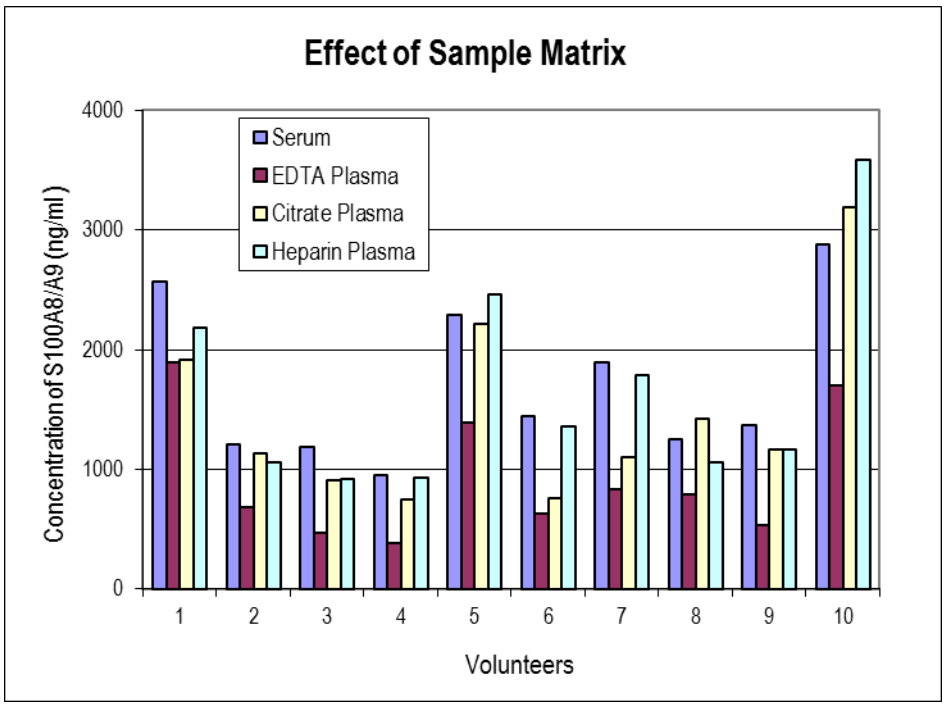

Figure 3: S100A8/A9 levels measured using Human S100A8/A9 ELISA from10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

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

Samples should be stored at –20°C. However, no decline in concentration of S100A8/A9 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 thimerosal, resulting in the final concentration of 0.03% and 0.01%, respectively.

Sample	*Incubation Temp, Period*	*Serum (ng/ml)*	*Plasma (ng/ml)*		
			EDTA	*Citrate*	*Heparin*
1	-20°C	1567	360	457	1353
	2-8°C, 1 day	1751	442	444	1312
	2-8°C, 7 days	1586	357	491	1458
2	-20°C	4326	1191	1223	3672
	2-8°C, 1 day	3655	1114	1188	3627
	2-8°C, 7 days	3784	1159	1244	2570
3	-20°C	2326	917	1058	3188
	2-8°C, 1 day	2291	1006	1256	3141
	2-8°C, 7 days	2405	876	1062	3070

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human S100A8/A9 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	3821	1941	2775	1911
	3x	4354	1388	2687	1801
	5x	4154	1493	2485	1739
2	1x	4066	1581	981	1605
	3x	3654	1723	1057	1613
	5x	3633	1774	1041	1513
3	1x	2609	822	866	1421
	3x	2551	776	890	1024
	5x	2302	761	784	1148

14. DEFINITION OF THE STANDARD

The recombinant human calprotectin (S100A8/A9) is used as the standard.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 150 unselected donors (83 men + 67 women) 22-65 years old were assayed with the BioVendor Human S100A8/A9 (Calprotectin) ELISA in our laboratory.

- **Age dependent distribution of S100A8/A9**

Sex	Age (years)	n	Mean	Median	SD	Min	Max
			S100A8/A9 (ng/ml)				
Men	21-29	13	2127	1833	1062	941	5027
	30-39	27	2311	2162	825	999	4260
	40-49	32	2189	1957	1006	644	4947
	50-59	4	2192	2174	271	1874	2546
	60-65	7	2657	2501	620	1757	3745
Women	22-29	12	2185	2139	610	1184	3257
	30-39	27	1982	1998	760	749	3492
	40-49	20	1927	1738	744	793	3481
	50-59	7	1557	1512	386	830	2033
	60-61	1	1227	1227	0	1227	1227

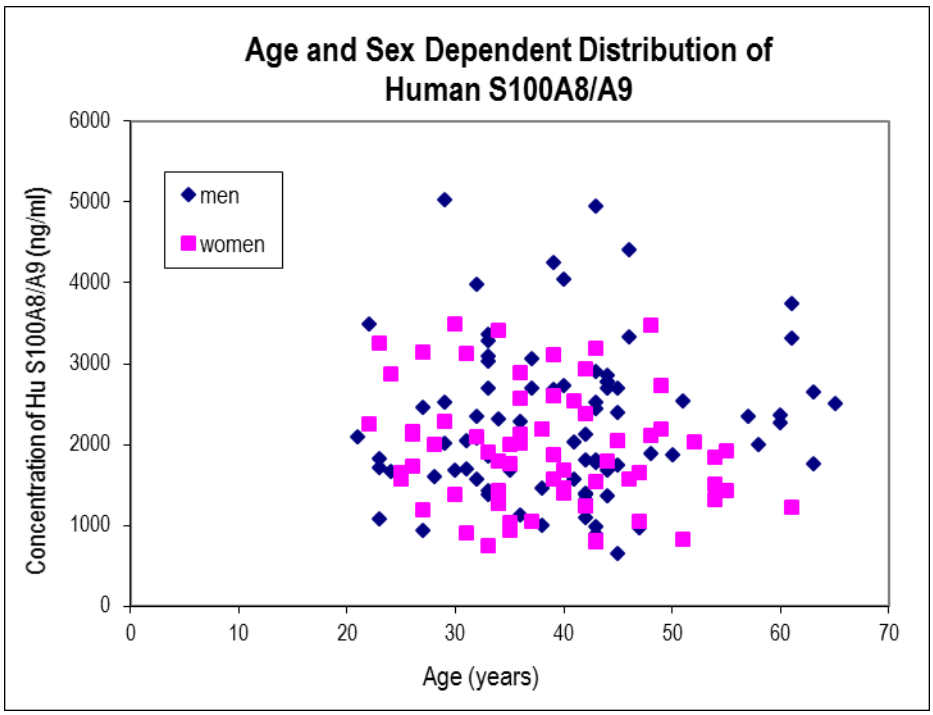

Figure 4: S100A8/A9 concentration plotted against donor age and sex.

- **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 S100A8/A9 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human S100A8/A9 (Calprotectin) ELISA was compared to two other commercial immunoassays by measuring 39 serum samples. The following correlation graphs were obtained:

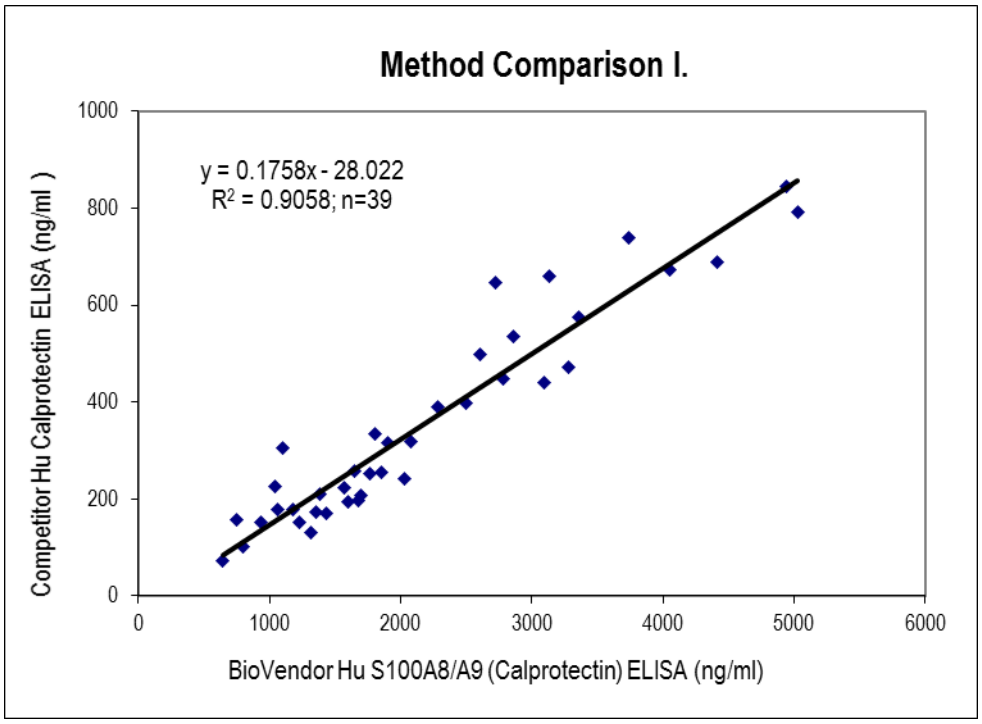

Figure 5: Method Comparison I.

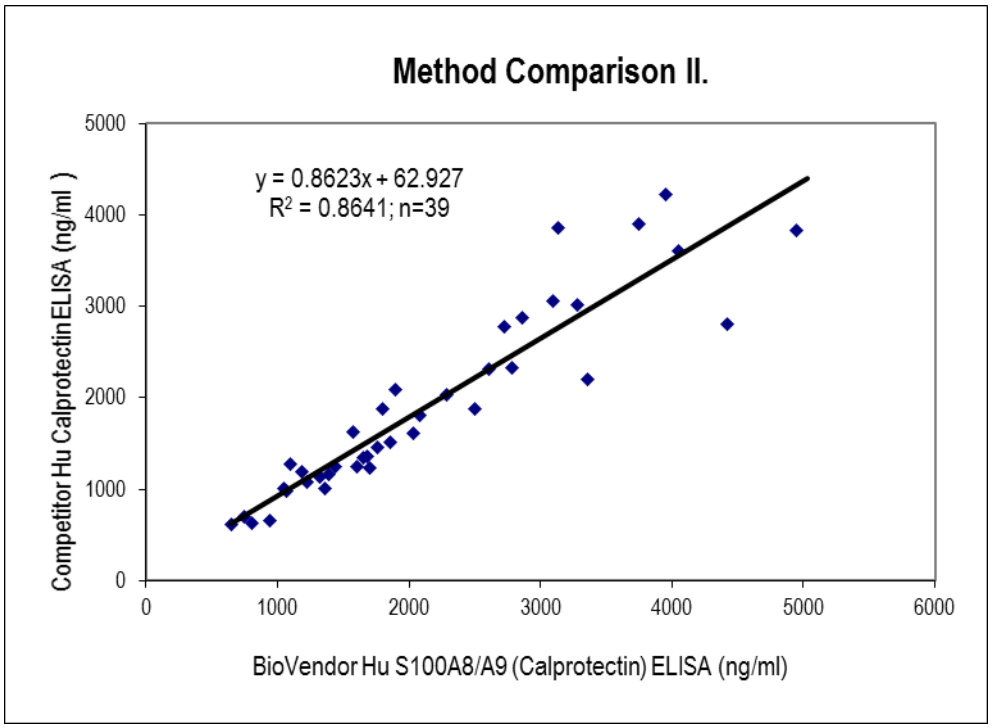

Figure 6: Method Comparison II.

17. TROUBLESHOOTING AND FAQS

›› Weak signal in all wells

Possible explanations:

- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

›› High signal and background in all wells

Possible explanations:

- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

›› High coefficient of variation (CV)

Possible explanation:

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

18. REFERENCES

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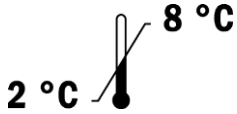
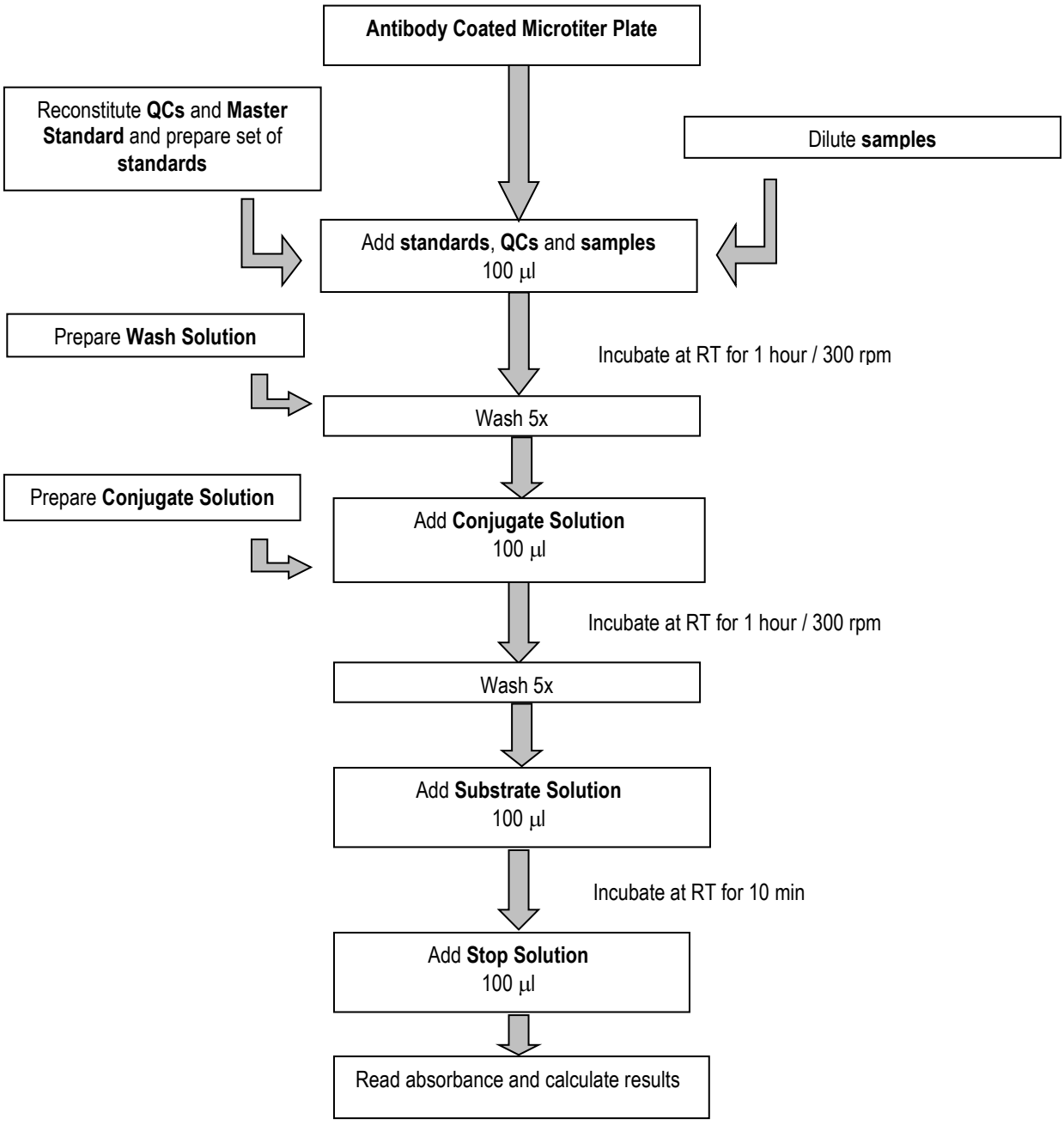
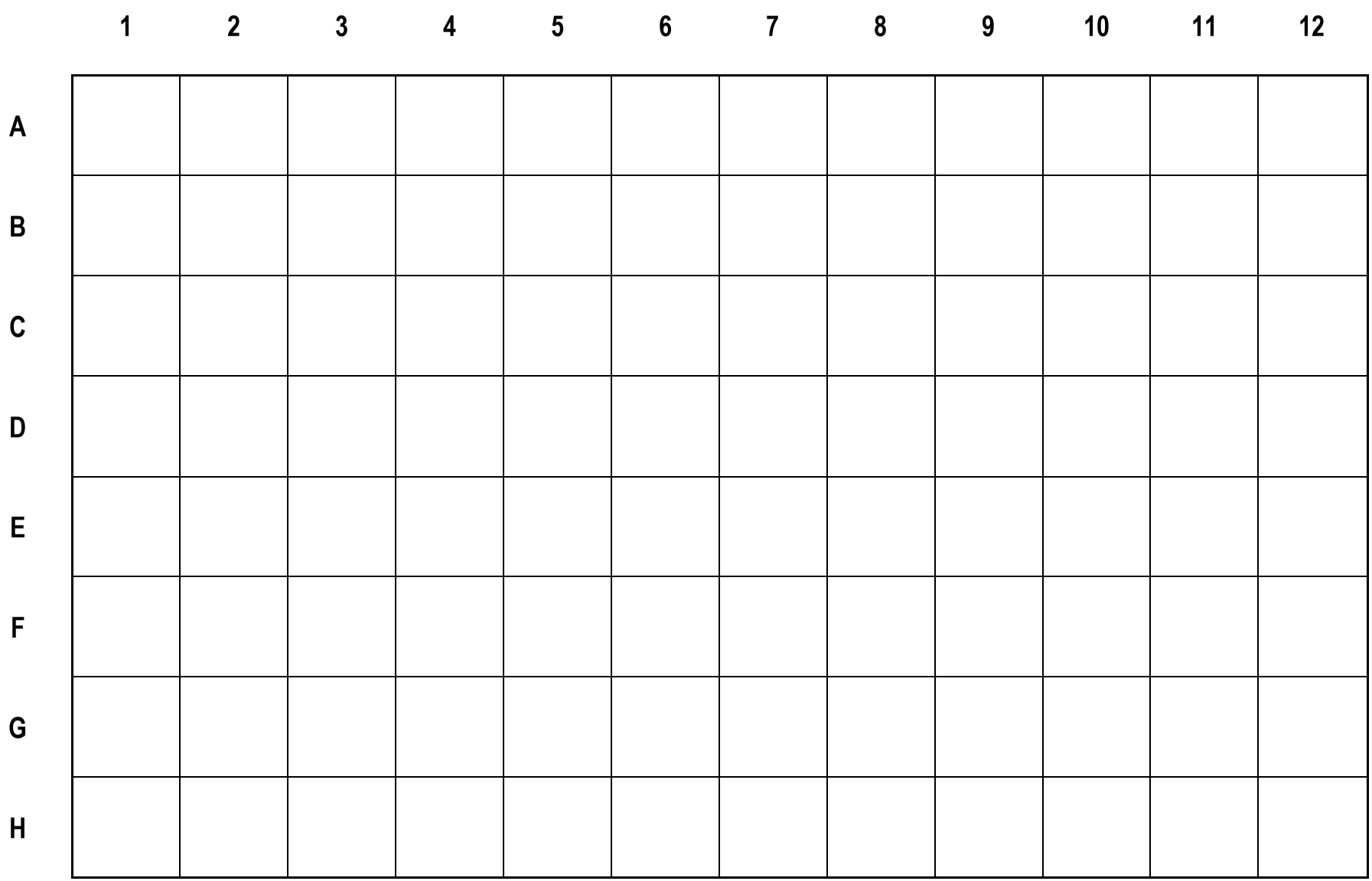
» For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

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

Assay Procedure Summary

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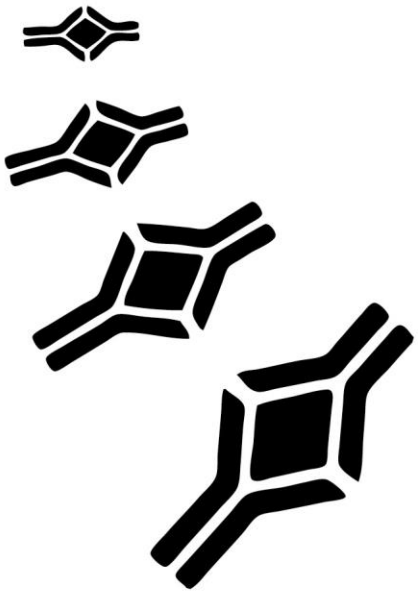

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