Instructions for Use

Lipocalin-2 / NGAL (Human) ELISA

EIA-5776

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(eng.008.A)

Please use only the valid version of the Instructions for Use provided with the kit.
Verwenden Sie nur die jeweils gültige, im Testkit enthaltene, Gebrauchsanweisung.
Si prega di usare la versione valida delle istruzioni per l'uso a disposizione con il kit.
Por favor, se usa solo la version valida de la metodico técnico incluido aqui en el kit.

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1 INTENDED USE

The Lipocalin-2 / NGAL (Human) ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human lipocalin-2.

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures lipocalin-2 in serum, plasma (EDTA), urine samples and stool extract
- 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 °C - 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

Lipocalin-2 (LCN2) is a 25 kDa secretory glycoprotein, also called NGAL (neutrophil gelatinase-associated lipocalin); NL (neutrophil lipocalin); p25; oncogen 24p3 and 25 kDa alpha-2-microglobulin-related subunit of MMP-9 (LCN2 forms a covalently linked, disulfide-bridged heterodimer with the 92 kDa type V collagenase (MMP-9)).

LCN2 is predominantly expressed in adipose tissue and liver. It belongs to the lipocalin superfamily that consists of over 20 small secretory proteins. Lipocalin-2 folds consist of 8 antiparallel β-sheets that surround a hydrophobic pocket. A common feature of this protein family, following from its structure, is its capacity to bind and transport small lipophilic substancies such as free fatty acids, retinoids, arachidonic acid and various steroids.

Although lipocalin-2-2 was identified more than a decade ago, the physiologic function of this protein remains poorly understood. LCN2 appears to be upregulated in cells under the “stress” (e.g. from infection, inflammation, in tissues undergoing involution to ischemia or neoplastic transformation).

Plasma levels of LCN2 rise in inflammatory or infective condition. It mediates an immune response to bacterial infection by sequestering iron. In this case, LCN2 may represent a promising candidate as a therapeutic agent against bacterial infection.

Several recent reports suggest that LCN2 might represent a sensitive biomarker for early renal injury. In cardiopulmonary bypass-induced acute renal injury and cisplatin-induced nephrotoxic injury, increased de novo synthesis of LCN2 in proximal tubule cells leads to sharply increased concentration of this protein in both urine and serum. LCN2 might also be critical for normal kidney formation in the earliest stages of mammalian development.

LCN2 may play an important role in breast cancer, in complex with MMP-9, by protecting MMP-9 from degradation thereby enhancing its enzymatic activity and facilitating angiogenesis and tumor growth. LCN2 is also highly expressed after malignant transformation of the lung, colon and pancreatic epithelia.

Circulating levels of LCN2 play a causative role in pathogenesis of obesity-induced metabolic disorders such as insulin resistance, Type 2 Diabetes mellitus and cardiovascular disorders. In addition, serum LCN2 concentrations were positively associated with adipocyte-fatty acid binding protein (A-FABP), a novel serum marker for adiposity and metabolic syndrome.

<u>Areas of investigation:</u>

Bacterial infection
Renal injury
Angiogenesis
Oncology
Diabetes mellitus
Metabolic syndrome

4 TEST PRINCIPLE

In the Lipocalin-2 / NGAL (Human) ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human lipocalin-2 antibody. After one hour incubation and washing, biotin labelled polyclonal anti-human lipocalin-2 antibody is added and incubated with captured lipocalin-2 for one hour. 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 lipocalin-2. 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 colorless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colorless until added to the plate. The color developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in color 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. (20x)	concentrated	0.70 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	20 mL
Biotin-Ab Diluent	ready to use	13 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 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.**
- **Centrifuge liquid containing microtube vials before opening.**
- **Always prepare only the appropriate quantity of reagents for your test.**
- **Do not use components after the expiration date marked on their label.**

9.1 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 °C - 8 °C and protected from the moisture.

Biotin-Ab Diluent

Streptavidin-HRP Conjugate

Substrate Solution

Stop Solution

Dilution Buffer

Stability and storage:

Opened reagents are stable 3 months when stored at 2 °C - 8 °C.

9.2 Assay reagents supplied concentrated or lyophilized

Human Lipocalin-2 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 lipocalin-2 in the stock solution is **10 ng/mL**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	10 ng/mL
300 µL of 10 ng/mL	300 µL	5 ng/mL
300 µL of 5 ng/mL	300 µL	2.5 ng/mL
300 µL of 2.5 ng/mL	300 µL	1.25 ng/mL
300 µL of 1.25 ng/mL	300 µL	0.6 ng/mL
300 µL of 0.6 ng/mL	300 µL	0.3 ng/mL

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

Stability and storage:

Reconstituted Master Standard must be used immediately or should be aliquoted and frozen at -20 °C for 3 months. Avoid repeating freezing/thawing 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!!!

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:

The reconstituted Quality Controls must be used immediately.

Do not store the reconstituted Quality Controls.

Note:

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

Biotin Labelled Antibody Conc. (20x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (20x) with 19 parts Biotin-Ab Diluent.

Example: 50 µL of Biotin Labelled Antibody Concentrate (20x) + 950 µL of Biotin-Ab Diluent for 1 strip (8 wells).

Stability and storage:

Opened Biotin Labelled Antibody Concentrate (20x) is stable 3 months when stored at 2 °C - 8 °C.

Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution.

Example: 100 mL of Wash Solution Concentrate (10x) + 900 mL of distilled water for use of all 96-wells.

Stability and storage:

The diluted Wash Solution is stable 1 month when stored at 2 °C - 8 °C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2 °C - 8 °C.

10 PREPARATION OF SAMPLES

The kit measures lipocalin-2 in serum, plasma (EDTA), urine samples and stool extracts.

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.

Serum samples:

Dilute serum or plasma samples 30x with Dilution Buffer just prior to the assay,

e.g. 5 µL of sample + 145 µL of Dilution Buffer when assaying samples as singlets, or preferably 10 µL of sample + 290 µL of Dilution Buffer for duplicates.

Mix well (not to foam). Vortex is recommended.

Urine samples:

For assaying urine samples, the dilution 10x is recommended,

e.g. 15 µL of urine sample + 135 µL of Dilution Buffer when assaying urine samples in singlets, or preferably 25 µL of urine sample + 225 µL of Dilution Buffer for duplicates.

Mix well (not to foam). Vortex is recommended.

Stool extract:

For protocol for preparation of stool extracts and other details, please contact us at drg@drg-diagnostics.de.

Recommended starting dilution for stool extract is 10x.

Stability and storage:

Samples have to be diluted just prior to the assay**.** 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 stability of serum and plasma samples when stored at 2 °C - 8 °C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of lipocalin-2.

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, 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 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 **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.
12. Stop the color development by adding **100 µL** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550 - 650 nm). Subtract readings at 630 nm (550 - 650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine lipocalin-2 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 10**	**Blank**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 5**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 2.5**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 1.25**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 0.6**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 0.3**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**QC HIGH**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC LOW**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

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 lipocalin-2 (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 calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 1.75 ng/mL (from standard curve) x 30 (dilution factor) = 52.5 ng/mL.

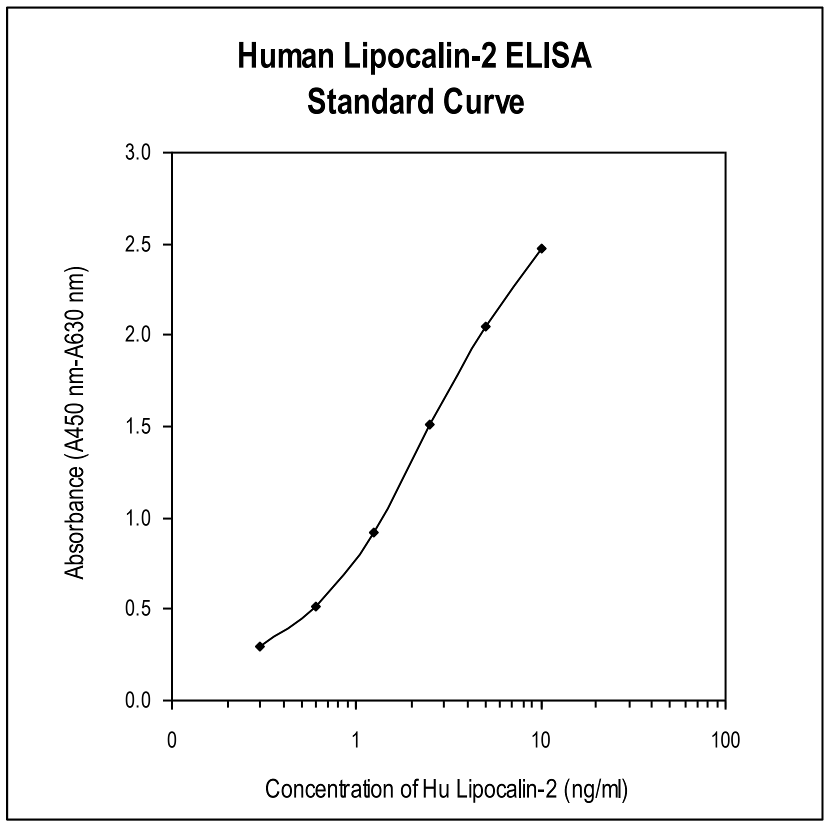

Figure 2: Typical Standard Curve for Human Lipocalin-2/NGAL ELISA.

13 PERFORMANCE CHARACTERISTICS

Typical analytical data of Lipocalin-2 / NGAL (Human) ELISA are presented in this chapter

13.1 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 lipocalin-2 values in wells and is 0.02 ng/mL.

*Dilution Buffer is pipetted into blank wells.

13.2 Limit of assay

Serum and plasma samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. Urine samples with absorbances exceeding the absorbance of the 2.5 ng/mL standard should be also measured again with higher dilution.

The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

13.3 Specificity

The antibodies used in this ELISA are specific for human lipocalin-2. No cross reactivity with mouse lipocalin-2 has been observed.

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

For details please contact us at drg@drg-diagnostics.de.

Mammalian serum sample	*Observed cross reactivity*
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

13.4 Precision

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

Sample	*Mean (ng/mL)*	*SD (ng/mL)*	*CV (%)*
1	68.2	4.8	7.0
2	23.6	2.0	8.4

Inter-assay (Run-to-Run) (n=7)

Sample	*Mean (ng/mL)*	*SD (ng/mL)*	*CV (%)*
1	32.6	3.2	9.8
2	38.1	3.7	9.8

13.5 Spiking Recovery

Serum samples were spiked with different amounts of human lipocalin-2 and assayed.

Sample	***O**bserved (ng/mL)*	***E**xpected (ng/mL)*	*Recovery **O/E** (%)*
1	11.0	-	-
	135.0	136.0	99.3
	66.5	73.5	90.5
	43.0	41.0	104.9
2	44.5	-	-
	159.5	169.5	94.1
	94.5	107.0	88.3
	82.5	74.5	110.7

13.6 Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/mL)*	***E**xpected (ng/mL)*	*Recovery **O/E** (%)*
1	-	74.4	-	-
	2x	39.6	37.2	106.5
	4x	20.4	18.6	109.7
	8x	9.3	9.3	100.0
2	-	48.3	-	-
	2x	25.8	24.2	106.8
	4x	12.0	12.1	99.4
	8x	6.3	6.0	104.3

13.7 Effect of sample matrix

EDTA, citrate and heparin plasmas were compared to respective serum samples from the same 10 individuals. However, we observed low correlation among lipocalin-2 values in serum and heparin and citrate plasma, respectively. Results are shown below:

Volunteer No.	*Serum (ng/mL)*	*Plasma (ng/mL)*		
		EDTA	*Citrate*	*Heparin*
1	36.3	34.5	24.8	42.6
2	47.0	48.8	31.8	45.8
3	63.1	66.3	43.4	48.8
4	33.1	28.0	26.1	29.1
5	35.4	32.3	24.9	46.6
6	47.3	48.3	40.0	42.4
7	43.2	35.7	18.7	31.5
Mean (ng/mL)	**43.6**	**42.0**	**30.0**	**41.0**
Mean Plasma/Serum (%)	**-**	**96.3**	**68.7**	**93.9**
Coefficient of determination (R^2)	**-**	**0.96**	**0.60**	**0.27**

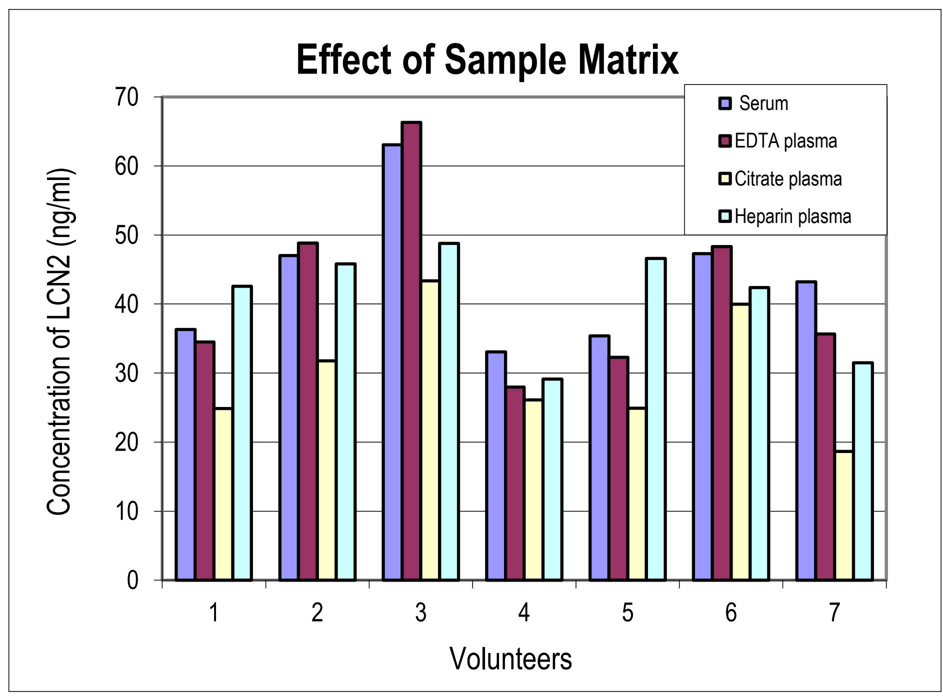

Figure 3: Lipocalin-2 levels measured using Human Lipocalin-2/NGAL ELISA in serum, EDTA, citrate and heparin plasma, respectively from the same 10 individuals.

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

Samples should be stored at -20 °C. However, no decline in concentration of lipocalin-2 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 0.05%, respectively.

Sample	*Incubation Temp. Period*	*Serum (ng/mL)*	*EDTA Plasma (ng/mL)*
1	-20 °C	57,6	51.0
	2 °C - 8 °C, 1 day	57.6	54.6
	2 °C - 8 °C, 7 days	58.8	48.8
2	-20 °C	48.0	52.8
	2 °C - 8 °C, 1 day	62.4	55.2
	2 °C - 8 °C, 7 days	59.4	52.8
3	-20 °C	55.8	28.2
	2 °C - 8 °C, 1 day	34.8	30.0
	2 °C - 8 °C, 7 days	31.2	21.0

13.9 Effect of Freezing/Thawing

No decline was observed in concentration of human lipocalin-2 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)*	*EDTA Plasma (ng/mL)*
1	1x	57,6	45.0
	3x	64.2	46.8
	5x	61.2	35.4
2	1x	59.4	58.2
	3x	79.2	48.6
	5x	75.6	37.8
3	1x	69.6	68.4
	3x	80.4	67.2
	5x	84.0	60.6

13.10 Diurnal Variation

Diurnal variation of lipocalin-2 levels in serum was determined in 4 patients in the course of 24 hours.

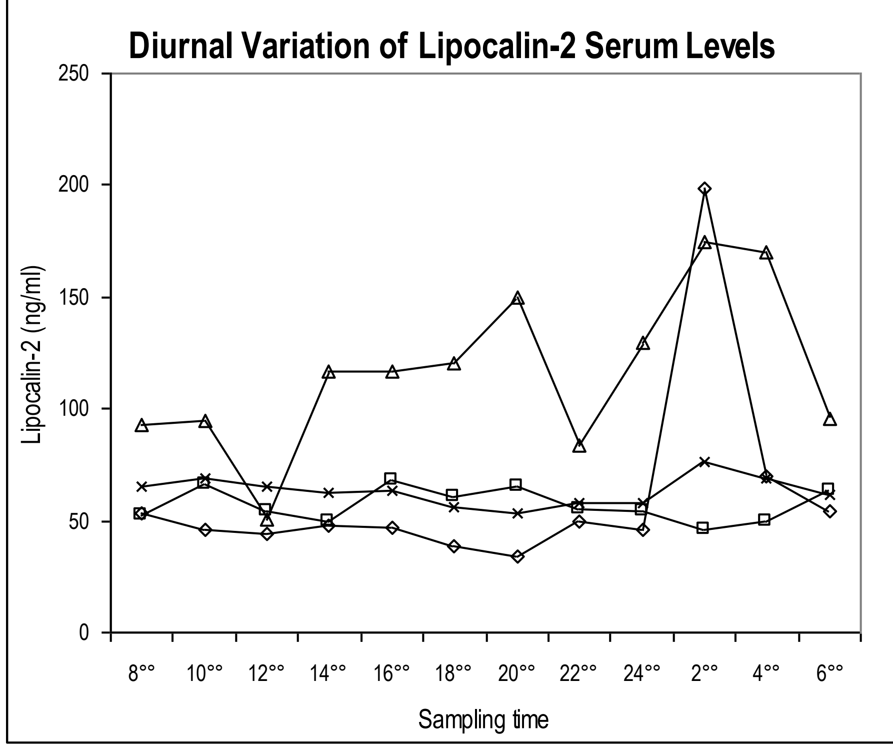

Figure. 4: Diurnal variation of serum lipocalin-2 levels.

14 DEFINITION OF THE STANDARD

The Standard used in this kit is a recombinant protein. The recombinant lipocalin-2 is 178 amino acid residues protein expressed in BL21 cells. The apparent molecular weight is 23 kDa.

15 PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum from 198 unselected donors (112 women + 86 men), 6-85 years old were assayed with Biovendor Human Lipocalin-2 /NGAL ELISA kit in our laboratory.

The presented data should be regarded only as guideline.

Age and sex dependent distribution of lipocalin-2

Sex	*Age (years)*	*n*	*Mean*	*SD*	*Min.*	*Max.*	*Median*
			lipocalin-2 (ng/mL)				
Men	13-18	6	43.4	21.3	17.4	87.0	39.9
	19-49	26	59.3	29.1	18.6	142.2	53.7
	50-85	54	62.1	33.1	14.4	169.2	53.7
Women	6-17	7	70,5	34.1	21.0	114.6	70.8
	24-50	39	79.8	43.2	13.8	251.4	70.8
	51-83	66	64.9	46.2	21.6	276.0	51.0

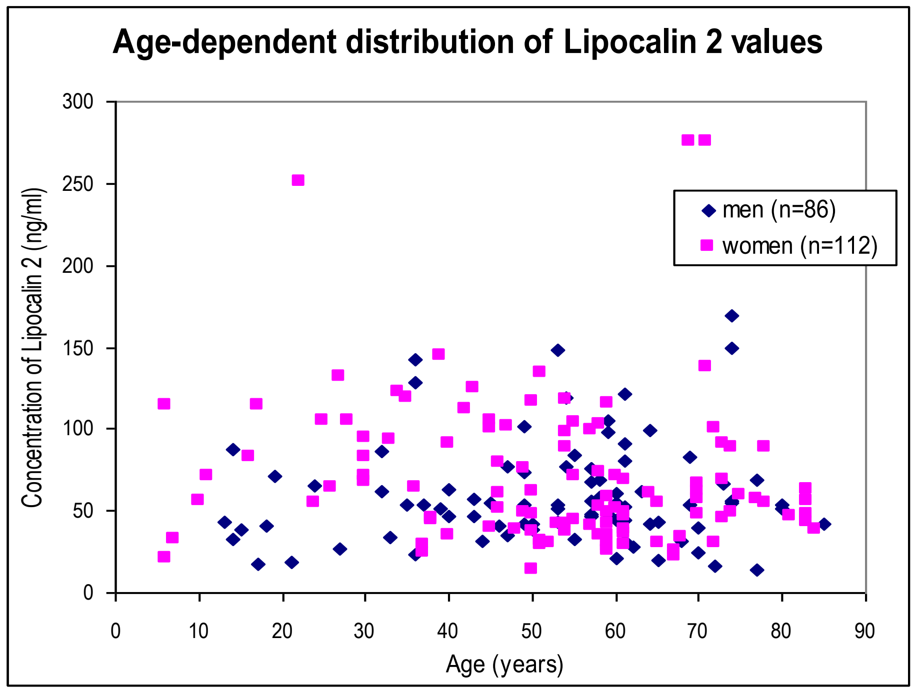

Figure 5: Lipocalin-2 concentration plotted against donor age.

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 references ranges for lipocalin-2 levels with the assay.

16 METHOD COMPARISON

The Lipocalin-2 / NGAL (Human) ELISA was compared to another commercial immunoassay by measuring 25 serum samples. The following correlation graph was obtained.

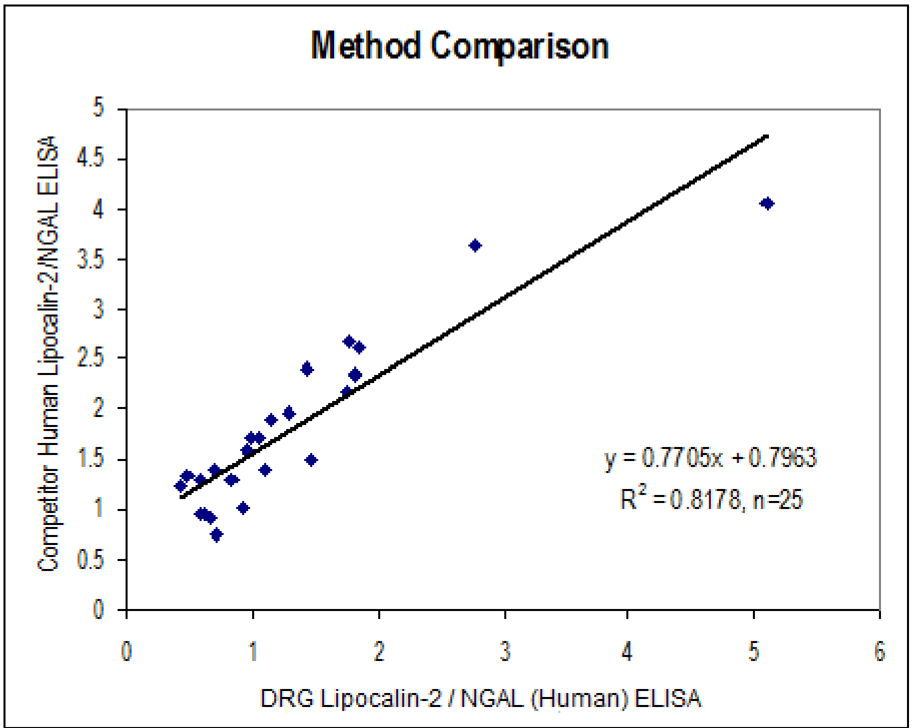

Figure 6: Method comparison.

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

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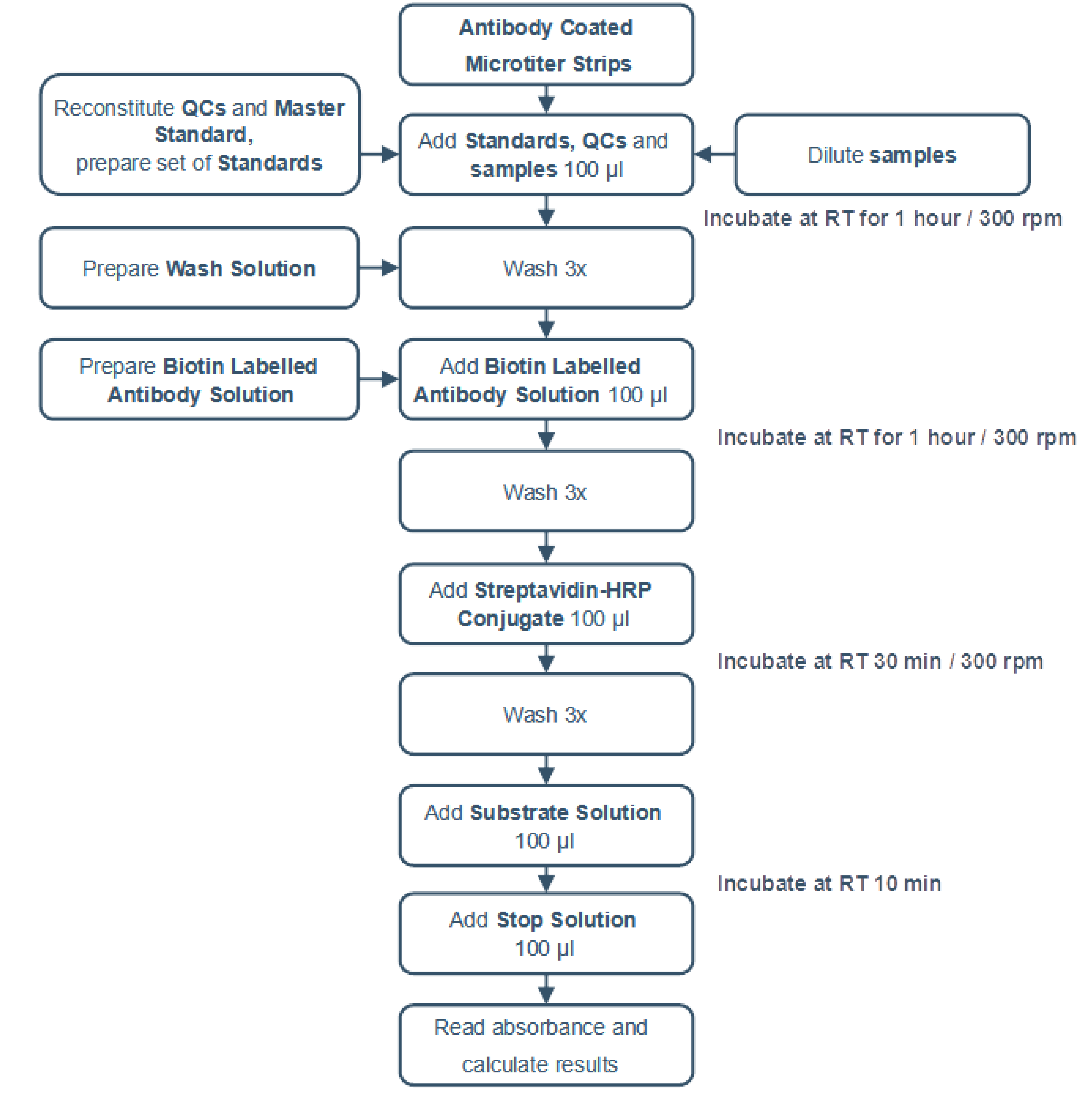
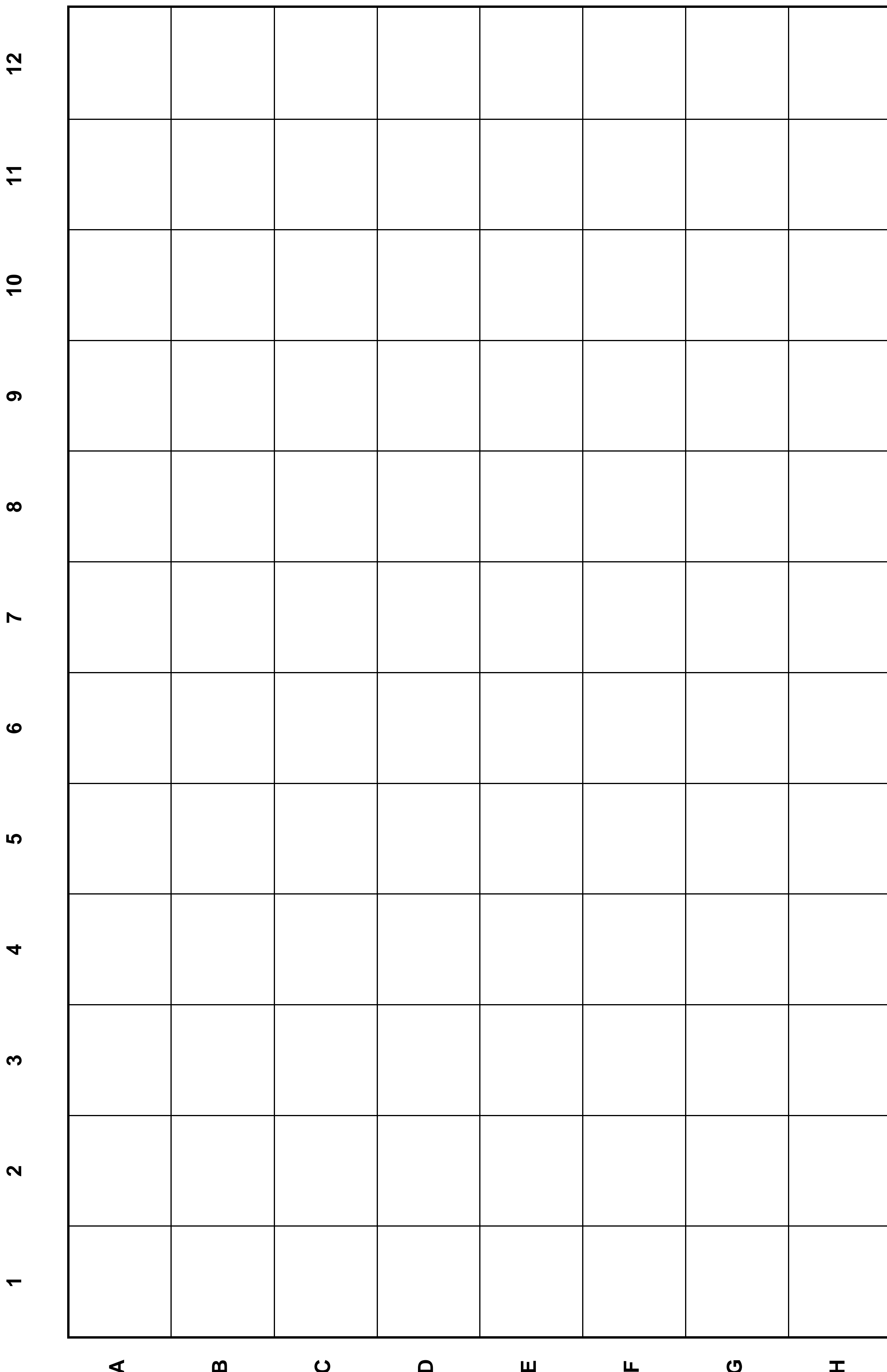

Antibody Coated Microtiter Strips
Reconstitute QCs and Master Standard, prepare set of Standards
Add Standards, QCs and samples 100 µl
Dilute samples
Incubate at RT for 1 hour / 300 rpm
Prepare Wash Solution
Wash 3x
Prepare Biotin Labelled Antibody Solution
Add Biotin Labelled Antibody Solution 100 µl
Incubate at RT for 1 hour / 300 rpm
Wash 3x
Add Streptavidin-HRP Conjugate 100 µl
Incubate at RT 30 min / 300 rpm
Wash 3x
Add Substrate Solution 100 µl
Incubate at RT 10 min
Add Stop Solution 100 µl
Read absorbance and calculate results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SYMBOLS USED

Symbol	English	Deutsch	Italiano	Español	Français
CE	European Conformity	CE-Konformitäts-kennzeichnung	Conformità europea	Conformidad europea	Conformité normes européennes
	Consult instructions for use *	Gebrauchsanweisung beachten *	Consultare le istruzioni per l'uso	Consulte las instrucciones de uso	Consulter les instructions d'utilisation
IVD	*In vitro* diagnostic medical device *	*In-vitro*-Diagnostikum *	Dispositivo medico-diagnostico in vitro	Producto sanitario para diagnóstico In vitro	Dispositif médical de diagnostic in vitro
REF	Catalogue number *	Artikelnummer *	Numero di Catalogo	Nûmero de catálogo	Référence de catalogue
LOT	Batch code *	Chargencode *	Codice del lotto	Codigo de lote	Numéro de lot
	Contains sufficient for <n> tests *	Ausreichend für <n> Prüfungen *	Contenuto sufficiente per "n" saggi	Contenido suficiente para <n> ensayos	Contenu suffisant pour "n" tests
	Temperature limit *	Temperaturbegrenzung *	Temperatura di conservazione	Temperatura de conservacion	Température de conservation
	Use-by date *	Verwendbar bis *	Utilizzare prima del	Establa hasta	Utiliser jusque
	Manufacturer *	Hersteller *	Fabbricante	Fabricante	Fabricant
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