BioVendor Group

ENG

Instructions for use:

HUMAN IMMUNOGLOBULIN FREE LIGHT CHAINS κ AND λ ELISA

Catalogue number:

RD194088100R

For research use only!

BioVendor R&D®

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CONTENT

HISTORY OF CHANGES

Previous version	Current Version
ENG.004.A	ENG.005.A
”History of changes” added.	
Chapter 9: A sentence „Centrifuge liquid containing microtube vials before opening“ added	

1. INTENDED USE

The RD194088100R Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA kit contains two sandwich enzyme immunoassays for the quantitative measurement of human immunoglobulin free light chains kappa and lambda.

Features

- **It is intended for research use only**
- The total assay time is less than 3.5 hours
- The kit measures FLC kappa and FLC lambda in serum, plasma (EDTA, citrate, heparin) and urine
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is human serum based. No animal sera are used
- 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 immunoglobulin molecules consist of two identical heavy chains which define immunoglobulin classes (IgG, IgA, IgM, IgD and IgE) and identical light chains (kappa or lambda) that are covalently linked to a heavy chain. In healthy individuals, the majority of light chains in serum exist bound to heavy chain. However, low levels of free light chains (FLCs) are found in serum of normal individuals due to their excess production over heavy chains by mature B-cells. In serum, FLC kappa exists predominantly as a monomer with a molecular weight of 22.5 kDa and FLC lambda as a dimer with a molecular weight of 45 kDa. This size difference results in a differential glomerular filtration rate and, consequently, a ratio of FLC kappa to FLC lambda of 1:1.6 in serum. FLCs are observed in urine too but filtration and reabsorption of low molecular proteins in the kidney strongly affects the FLC concentration so that urinary FLC level is low in healthy individuals.
FLC are a natural product of B lymphocytes and, as such, represent a unique biomarker of neoplastic and reactive B cell-related disorders. Increased FLCs are associated with malignant plasma dyscrasia and other lymphocyterelated immunoproliferative disorders. The detection of the FLCs is important diagnostic aid for a variety of monoclonal gammopathies, such as multiple myeloma, Waldenstrom macroglobulinemia, nonsecretory myeloma, smoldering multiple myeloma, monoclonal gammopathy of undetermined significance. Accurate measurement of monoclonal free light chains in serum and/or urine is especially important in light-chain diseases, such as light-chain myeloma, primary systemic amyloidosis, and light chain-deposition disease. The ability to quantify monoclonal FLCs may be useful to monitor the disease. In patients with light chain myeloma, either of light chain, kappa or lambda, is dominantly produced and resulting in marked changes of the FLC κ/λ ratio in the early phase of the disease. The detection of urinary monoclonal kappa or lambda free light chains of immunoglobulin, also known as Bence Jones proteins (BJP), are important for identifying and monitoring B-cell malignancies too.
In addition, compared with the healthy state, the synthesis of polyclonal FLC is markedly increased in conditions associated with B cell activation as found in certain inflammatory or autoimmune diseases (e.g. systemic lupus erythematosus, rheumatoid arthritis, or multiple sclerosis, as well as in cancer, diabetes mellitus, and AIDS).

Areas of investigation:
Oncology
B cell-related disorder
Autoimmune diseases

4. TEST PRINCIPLE

In the BioVendor Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with monoclonal anti-human immunoglobulin FLC kappa or FLC lambda antibody. After 60 minutes incubation and washing, biotin labelled second monoclonal antibody is added and incubated with captured antibody-FLC kappa or lambda complex 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 FLC. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.
Test principle is the same for both ELISAs.

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
Human FLC kappa Antibody Coated Microtiter Strips	ready to use	96 wells
Human FLC kappa Biotin Labelled Antibody Conc. (30x)	concentrated	0.45 ml
Human FLC kappa Master Standard	lyophilized	2 vials
Human FLC kappa Quality Control HIGH	lyophilized	2 vials
Human FLC kappa Quality Control LOW	lyophilized	2 vials
Human FLC lambda Antibody Coated Microtiter Strips	ready to use	96 wells
Human FLC lambda Biotin Labelled Antibody Conc. (30x)	concentrated	0.45 ml
Human FLC lambda Master Standard	lyophilized	2 vials
Human FLC lambda Quality Control HIGH	lyophilized	2 vials
Human FLC lambda Quality Control LOW	lyophilized	2 vials
Streptavidin-HRP Conjugate Conc. (30x)	concentrated	1.0 ml
Dilution Buffer Conc. (10x)	concentrated	22 ml
Wash Solution Conc. (10x)	concentrated	2 x 100 ml
Substrate Solution	ready to use	2 x 13 ml
Stop Solution	ready to use	2 x 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.

Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips (FLC kappa or lambda)

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desicant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

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:

Dilution Buffer Conc. (10x)

Dilute Dilution Buffer Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution. Example: 20 ml of Dilution Buffer Concentrate (10x) + 180 ml of distilled water for use of all 96-wells.
Dilution Buffer is same for dilution of FLC Kappa and Lambda Standards, Quality Controls, samples, Biotin Labelled Antibody Concentrates and Streptavidin-HRP Conjugate Concentrate.

Stability and storage:
The diluted Dilution Buffer is stable 1 month when stored at 2-8°C. Opened Dilution Buffer Concentrate (10x) is stable 3 months when stored at 2-8°C.

FLC kappa 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 FLC kappa in the stock solution is **320 µg/l**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	320 µg/l
250 µl of stock	250 µl	160 µg/l
250 µl of 160 µg/l	250 µl	80 µg/l
250 µl of 80 µg/l	250 µl	40 µg/l
250 µl of 40 µg/l	250 µl	20 µg/l
250 µl of 20 µg/l	250 µl	10 µg/l

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

Stability and storage:
Do not store the Standard stock solution and set of standards.

FLC kappa 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:
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 IFU and CoA and that ELISA test was carried out properly.

FLC kappa Biotin Labelled Antibody Conc. (30x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (30x) with 29 parts Dilution Buffer.
Example: 33 µl of Biotin Labelled Antibody Concentrate (30x) + 957 µl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (30x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

LC lambda 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 FLC lambda in the stock solution is **560 µg/l**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	560 µg/l
250 µl of stock	250 µl	280 µg/l
250 µl of 280 µg/l	250 µl	140 µg/l
250 µl of 140 µg/l	250 µl	70 µg/l
250 µl of 70 µg/l	250 µl	35 µg/l
250 µl of 35 µg/l	250 µl	17.5 µg/l

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

Stability and storage:
Do not store Standard stock solution and set of standards.

FLC lambda 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:
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 IFU and CoA and that ELISA test was carried out properly.

FLC lambda Biotin Labelled Antibody Conc. (30x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (30x) with 29 parts Dilution Buffer.
Example: 33 µl of Biotin Labelled Antibody Concentrate (30x) + 957 µl of Dilution Buffer for 1 strip (8 wells).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (30x) is stable 3 months when stored at 2-8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Streptavidin-HRP Conjugate Conc. (30x)

Prepare the working Streptavidin-HRP Conjugate solution by adding 1 part Streptavidin-HRP Conjugate Concentrate (30x) with 29 parts Dilution Buffer. Example: 33 µl of Streptavidin-HRP Conjugate Concentrate (30x) + 957 µl of Dilution Buffer for 1 strip (8 wells).
Streptavidin-HRP Conjugate solution is same for both ELISAs.

Stability and storage:
Opened Streptavidin-HRP Conjugate Concentrate (30x) is stable 3 months when stored at 2-8°C.

Do not store the diluted Streptavidin-HRP 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 FLC kappa and FLC lambda in serum, plasma (EDTA, citrate, heparin) and urine.

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.

Dilution factor of samples is the same for both ELISAs and therefore dilute samples in enough amounts for both assays.

Serum or plasma samples:

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

Results exceeding FLC kappa level of 320 µg/l or FLC lambda level of 560 µg/l should by repeated with more diluted samples. It is recommended to dilute samples just prior to assay 2 000-fold and 20 000-fold in next assay as follows (for duplicates and singlets):
50 µl of dilution 200-fold + 450 µl of Dilution Buffer for final dilution 2 000-fold
5 µl of dilution 200-fold + 495 µl of Dilution Buffer for final dilution 20 000-fold
Dilution factor needs to be taken into consideration in calculating of the FLC kappa or FLC lambda concentration.

Urine samples:

Dilute urine samples from healthy 40x with Dilution Buffer just prior to the assay, e.g. 15 µl of sample + 585 µl of Dilution Buffer, and for patient 200x, e.g. 5 µl of sample + 995 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Serum and plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage. Urine 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 FLC concentration.

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 1a and Figure 1b 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 solution into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 min**, 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 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 FLC 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 320**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 160**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 80**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 40**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 20**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 10**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1a: Example of a work sheet for FLC kappa ELISA.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 560**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 280**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 140**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 70**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 35**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 17.5**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1b: Example of a work sheet for FLC lambda ELISA.

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 FLC µg/l 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. 100 µg/l (from standard curve) x 200 (dilution factor) = 20 000 µg/l = 20 mg/l.

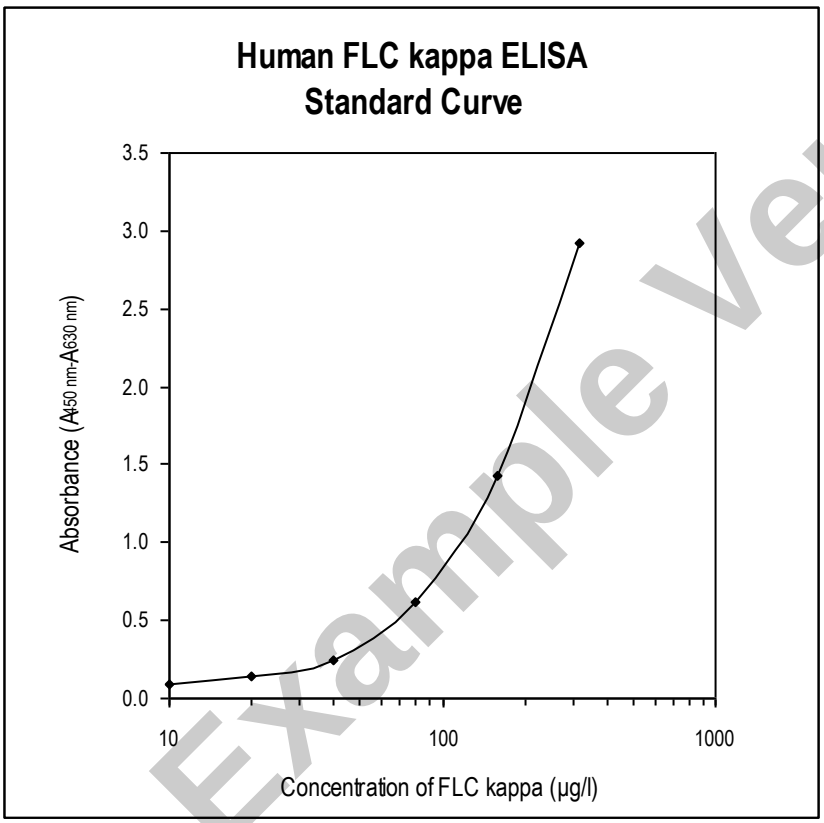

Figure 2a: Typical Standard Curve for FLC kappa ELISA.

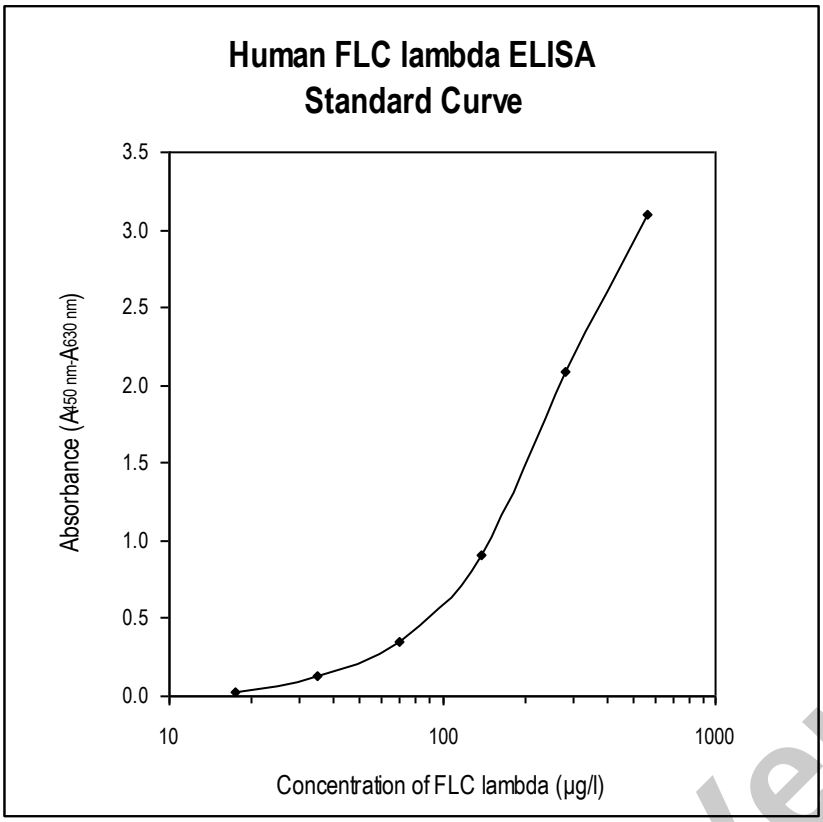

Figure 2b: Typical Standard Curve for FLC lambda ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA are presented in this chapter.

FLC kappa ELISA

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 FLC kappa values in wells and is 6 µg/l.
*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 FLC kappa.

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
Goat	no
Horse	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 (mg/l)	SD (mg/l)	CV (%)
1	7.2	0.30	4.0
2	11.2	0.22	2.0

Inter-assay (Run-to-Run) (n=8)

Sample	Mean (mg/l)	SD (mg/l)	CV (%)
1	7.8	0.54	7.0
2	11.9	0.82	6.9

Spiking Recovery

Serum samples were spiked with different amounts of FLC kappa and assayed.

Sample	Observed (mg/l)	Expected (mg/l)	Recovery O/E (%)
1	6.2	-	-
	21.3	22.2	95.9
	14.9	14.2	104.9
	10.7	10.2	104.9
2	14.2	-	-
	30.5	30.2	101.1
	22.6	22.2	101.8
	18.1	18.2	99.5

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (mg/l)	Expected (mg/l)	Recovery O/E (%)
1	-	32.14	-	-
	2x	14.54	16.07	90.5
	4x	7.16	8.04	89.1
	8x	3.56	4.02	88.6
2	-	54.14	-	-
	2x	22.66	27.08	83.7
	4x	11.52	13.54	85.1
	8x	6.06	6.77	89.5

FLC lambda ELISA

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 FLC lambda values in wells and is 5 µg/l.
***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 FLC lambda.

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
Goat	no
Horse	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 (mg/l)	SD (mg/l)	CV (%)
1	15.0	0.90	6.0
2	25.0	0.75	3.0

Inter-assay (Run-to-Run) (n=8)

Sample	Mean (mg/l)	SD (mg/l)	CV (%)
1	16.8	1.14	6.8
2	24.7	1.47	5.9

Spiking Recovery

Serum samples were spiked with different amounts of FLC lambda and assayed.

Sample	Observed (mg/l)	Expected (mg/l)	Recovery O/E (%)
1	9.20	-	-
	38.98	37.20	104.8
	23.84	23.20	102.8
	15.78	16.20	97.4
2	18.06	-	-
	30.34	30.06	100.9
	23.38	24.06	97.2
	21.54	21.06	102.2

Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (mg/l)	Expected (mg/l)	Recovery O/E (%)
1	-	63.00	-	-
	2x	27.78	31.50	88.2
	4x	14.32	15.75	90.9
	8x	6.50	7.88	82.5
2	-	84.40	-	-
	2x	36.38	42.20	86.2
	4x	17.76	21.10	84.2
	8x	8.74	10.55	82.8

FLC kappa ELISA and FLC lambda ELISA

Effect of sample matrix

Heparin, citrate and EDTA plasmas were compared to respective serum samples from the same 10 individuals. Results are shown below:

FLC kappa				
Volunteer No.	Serum (mg/l)	Plasma (mg/l)		
		EDTA	Citrate	Heparin
1	13.05	13.14	12.08	12.75
2	23.82	18.69	19.05	21.18
3	15.36	15.18	15.92	14.09
4	12.51	14.16	12.77	14.69
5	10.86	9.90	7.26	10.70
6	7.77	7.94	6.39	8.24
7	11.54	10.04	10.00	10.92
8	10.06	10.16	9.36	8.76
9	4.42	3.92	3.72	4.30
10	10.26	10.10	9.00	10.46
Mean (mg/l)	12.02	11.36	10.55	11.36
Mean plasma/serum (%)	-	94.5	87.7	96.6
Coefficient of determination R^2	-	0.903	0.903	0.939

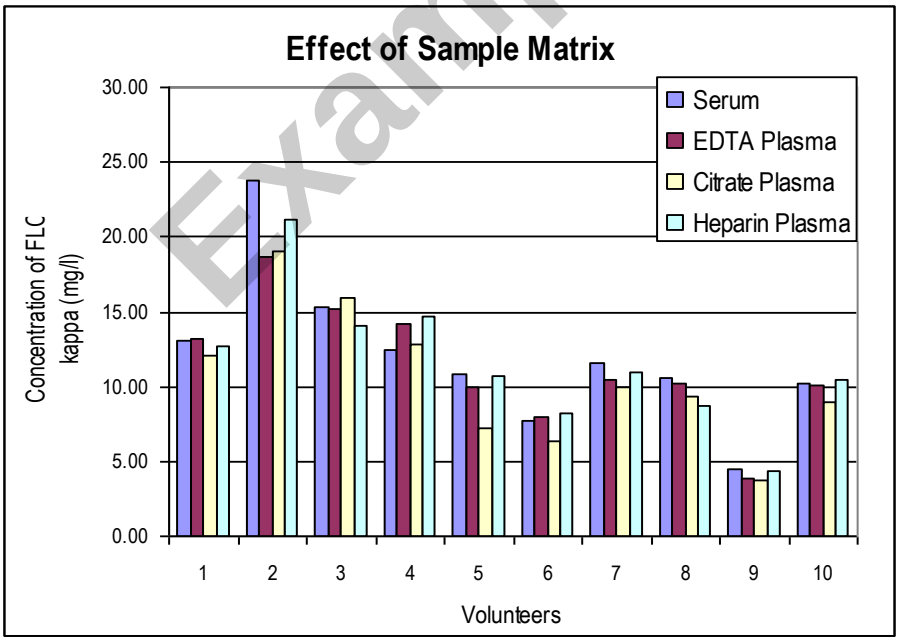

Figure 3a: FLC kappa levels measured using Human Immunoglobulin Free Light Chains Kappa ELISA from10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

FLC lambda				
Volunteer No.	Serum (mg/l)	Plasma (mg/l)		
		EDTA	Citrate	Heparin
1	14.99	17.31	12.45	14.40
2	31.70	28.31	22.98	26.43
3	10.74	12.86	9.95	8.43
4	12.99	15.02	11.45	14.57
5	11.52	10.64	6.92	11.31
6	10.02	11.01	7.23	10.67
7	13.60	13.58	11.64	13.44
8	7.76	9.18	6.74	5.94
9	14.64	14.60	11.9	14.88
10	10.58	10.10	9.06	11.00
Mean (mg/l)	**13.85**	**14.26**	**11.03**	**13.21**
Mean plasma/serum (%)	**-**	**102.9**	**79.6**	**95.4**
Coefficient of determination R^2	**-**	**0.95**	**0.945**	**0.923**

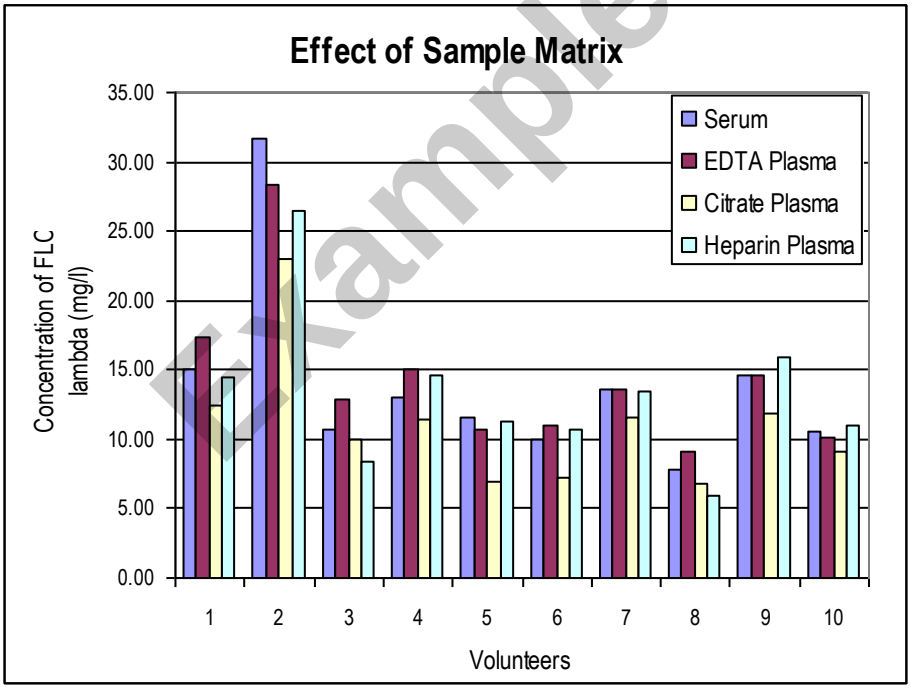

Figure 3b: FLC lambda levels measured using Human Immunoglobulin Free Light Chains *Lambda ELISA from10 individuals using serum, EDTA, citrate and heparin plasma, respectively.*

FLC kappa/lambda ratio				
Volunteer No.	Serum	Plasma		
		EDTA	Citrate	Heparin
1	0.87	0.76	0.97	0.89
2	0.75	0.66	0.83	0.8
3	1.43	1.18	1.60	1.67
4	0.96	0.94	1.12	1.01
5	0.94	0.93	1.05	0.95
6	0.78	0.72	0.88	0.77
7	0.85	0.77	0.86	0.81
8	1.37	1.11	1.39	1.47
9	0.3	0.27	0.31	0.27
10	0.97	1.00	0.99	0.95
Mean	**0.92**	**0.83**	**1.00**	**0.96**
Mean plasma/serum (%)	**-**	**90.2**	**108.6**	**104.3**
Coefficient of determination R^2	**-**	**0.925**	**0.975**	**0.981**

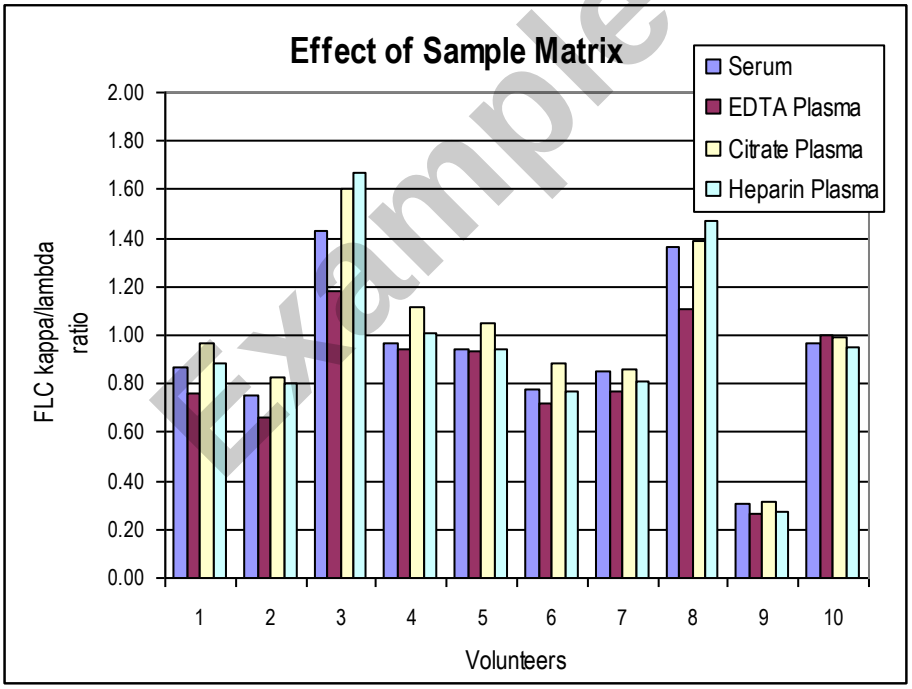

Figure 3c: FLC kappa/lambda ratio measured using Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA from10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

Stability of samples stored at 2-8°C

Samples should be stored at –20°C. However, no decline in concentration of FLC kappa, FLC lambda and FLC kappa/lambda ratio were 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.

FLC kappa					
Sample	Incubation Temp., Period	Serum (mg/l)	Plasma (mg/l)		
			EDTA	Citrate	Heparin
1	-20°C	11.54	10.40	10.00	10.92
	2-8°C, 1 day	11.18	11.06	10.36	10.76
	2-8°C, 7 days	11.64	10.24	10.38	11.38
2	-20°C	10.60	10.16	9.36	8.76
	2-8°C, 1 day	10.90	10.00	10.14	10.84
	2-8°C, 7 days	9.38	10.04	9.04	10.54
3	-20°C	4.42	3.92	3.72	4.30
	2-8°C, 1 day	4.48	3.96	3.92	4.44
	2-8°C, 7 days	3.84	3.20	3.16	3.62

FLC lambda					
Sample	Incubation Temp., Period	Serum (mg/l)	Plasma (mg/l)		
			EDTA	Citrate	Heparin
1	-20°C	13.60	13.58	11.64	13.44
	2-8°C, 1 day	12.72	13.68	11.80	13.04
	2-8°C, 7 days	13.82	13.82	12.66	13.80
2	-20°C	7.76	9.18	6.74	5.94
	2-8°C, 1 day	8.16	10.08	6.94	8.60
	2-8°C, 7 days	6.64	10.32	7.18	7.76
3	-20°C	14.64	14.60	11.90	15.88
	2-8°C, 1 day	15.80	13.96	12.66	15.62
	2-8°C, 7 days	14.88	14.24	11.78	14.58

FLC kappa/lambda ratio					
Sample	Incubation Temp., Period	Serum (mg/l)	Plasma (mg/l)		
			EDTA	Citrate	Heparin
1	-20°C	0.85	0.77	0.86	0.81
	2-8°C, 1 day	0.88	0.81	0.88	0.83
	2-8°C, 7 days	0.84	0.74	0.82	0.82
2	-20°C	1.37	1.11	1.39	1.47
	2-8°C, 1 day	1.34	0.99	1.46	1.26
	2-8°C, 7 days	1.41	0.97	1.26	1.20
3	-20°C	0.30	0.27	0.31	0.27
	2-8°C, 1 day	0.28	0.28	0.31	0.28
	2-8°C, 7 days	0.26	0.22	0.27	0.25

Effect of Freezing/Thawing

No decline was observed in concentration of human FLC kappa, FLC lambda and FLC kappa/lambda ratio in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

FLC kappa					
Sample	Number of f/t cycles	Serum (mg/l)	Plasma (mg/l)		
			EDTA	Citrate	Heparin
1	1x	13.52	14.19	12.32	13.24
	3x	13.39	14.52	11.46	14.18
	5x	13.12	13.18	12.52	11.04
2	1x	9.04	9.18	8.74	8.60
	3x	9.36	8.82	7.86	9.30
	5x	8.10	8.42	8.58	6.22
3	1x	10.08	10.02	7.90	10.14
	3x	9.14	9.68	8.38	8.84
	5x	9.76	9.90	7.76	9.62

FLC lambda					
Sample	Number of f/t cycles	Serum (mg/l)	Plasma (mg/l)		
			EDTA	Citrate	Heparin
1	1x	17.52	20.96	14.48	17.92
	3x	16.72	21.02	14.22	17.04
	5x	17.48	20.20	14.30	14.78
2	1x	12.19	12.02	11.08	11.44
	3x	12.02	12.10	10.78	12.08
	5x	11.96	12.56	10.44	7.58
3	1x	27.14	33.58	23.02	28.08
	3x	28.50	29.02	22.38	28.10
	5x	28.16	30.28	22.90	28.66

FLC kappa/lambda ratio					
Sample	Number of f/t cycles	Serum (mg/l)	Plasma		
			EDTA	Citrate	Heparin
1	1x	0.77	0.71	0.85	0.74
	3x	0.80	0.69	0.81	0.83
	5x	0.75	0.65	0.88	0.75
2	1x	0.74	0.76	0.79	0.75
	3x	0.78	0.73	0.73	0.77
	5x	0.68	0.67	0.82	0.82
3	1x	0.37	0.30	0.34	0.36
	3x	0.32	0.33	0.37	0.31
	5x	0.35	0.33	0.34	0.34

14. DEFINITION OF THE STANDARD

Standard in these both assays is human serum based. For evaluation of standards see chapter 16.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 40 unselected donors 41-64 years old were assayed with the Biovendor Human Immunoglobulin Free Light Chains Kappa and Lambda ELISA in external laboratory.

Normal value

FLC kappa normal value (mean +/- SEM) is 11.5 +/- 0.58 mg/l
FLC kappa normal range (mean +/- 2xSD) is 11.5 +/- 6.72 mg/l

FLC lambda normal value (mean +/- SEM) is 17.39 +/- 1.08 mg/l
FLC lambda normal range (mean +/- 2xSD) is 17.39 +/- 13.68 mg/l

FLC kappa/lambda ratio normal value (mean +/- SEM) is 0.707 +/- 0.037
FLC kappa/lambda ratio normal range (mean +/- 2xSD) is 0.707 +/- 0.466

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 sample in the assay. Each laboratory should establish its own normal and pathological references ranges for FLCs levels with the assay.

The following results were obtained when serum from 160 unselected blood donors (80 Women + 80 Men) 5-86 years old were assayed with BioVendor Human Immunoglobulin Free Light Chain Kappa and Lambda ELISA kit in our laboratory.

Age and sex dependent distribution of FLCs

	FLC kappa (mg/l)			FLC lambda (mg/l)			FLC kappa/lambda		
	Women n=80	Men n=80	**W + M n=160**	Women n=80	Men n=80	**W + M n=160**	Women n=80	Men n=80	**W + M n=160**
Mean	11.15	10.31	**10.73**	16.56	17.22	**16.89**	0.685	0.645	**0.665**
Median	9.52	8.70	**9.30**	14.55	14.51	**14.55**	0.649	0.595	**0.614**
SD	6.23	5.69	**5.98**	6.79	9.49	**8.29**	0.287	0.300	**0.295**
Min.	3.15	2.25	**2.25**	4.58	7.35	**4.58**	0.230	0.244	**0.230**
Max.	34.35	31.50	**34.35**	43.43	54.23	**54.23**	1.761	1.852	**1.852**

Age (years)	W+M n	Mean	SD	Min.	Max.
		FLC kappa (mg/l)			
5-19	12	6.15	3.38	2.25	13.95
20-29	21	7.42	3.08	3.15	17.25
30-39	20	8.01	2.47	3.15	11.85
40-49	31	10.44	4.50	3.45	20.55
50-59	33	10.16	3.32	5.25	16.20
60-69	21	14.66	7.95	3.15	31.50
70-79	14	14.70	6.16	6.00	28.20
80-86	8	19.22	9.03	6.15	34.35

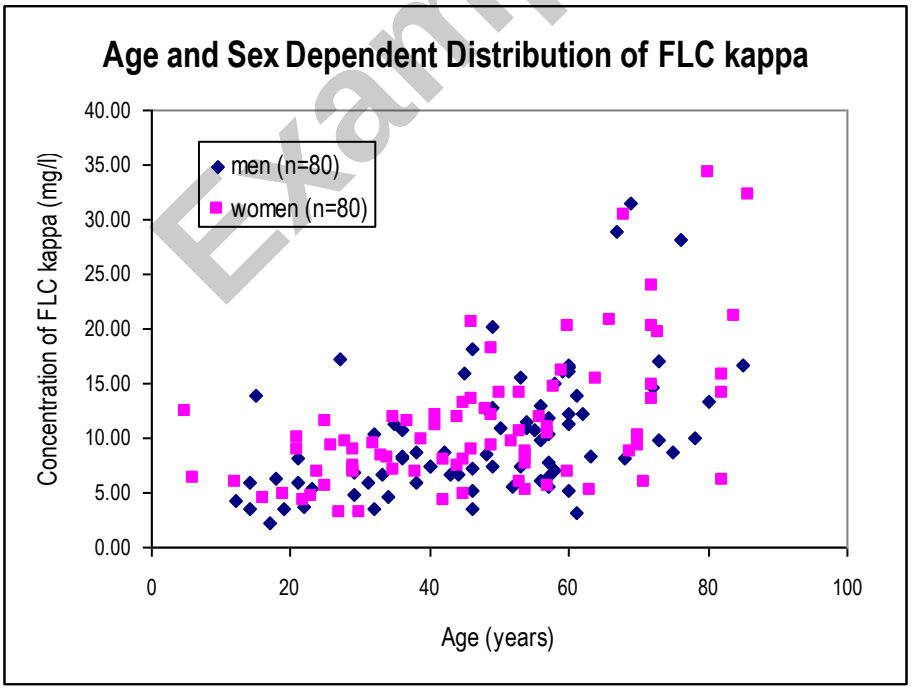

Figure 4a: FLC kappa concentration plotted against donor age.

Age (years)	W+M n	Mean	SD	Min.	Max.
		FLC lambda (mg/l)			
5-19	12	11.49	3.48	7.35	19.50
20-29	21	13.59	2.85	9.68	21.80
30-39	20	16.60	6.09	7.52	32.18
40-49	31	18.10	9.43	4.58	49.80
50-59	33	15.10	5.59	6.30	29.70
60-69	21	21.01	11.52	8.25	54.23
70-79	14	19.69	9.24	12.15	48.45
80-86	8	21.34	9.54	9.00	38.85

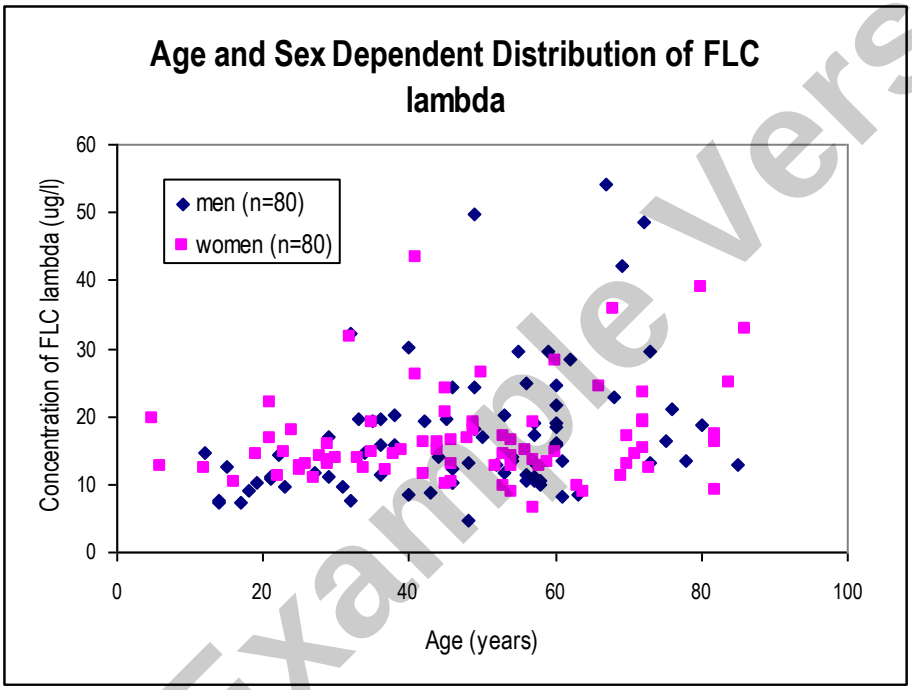

Figure 4b: FLC lambda concentration plotted against donor age.

Age (years)	W+M n	Mean	SD	Min.	Max.
		FLC kappa/lambda			
5-19	12	0.536	0.228	0.284	1.101
20-29	21	0.560	0.258	0.290	1.474
30-39	20	0.515	0.183	0.230	0.814
40-49	31	0.658	0.331	0.244	1.852
50-59	33	0.710	0.237	0.359	1.408
60-69	21	0.741	0.318	0.355	1.033
70-79	14	0.811	0.351	0.300	1.617
80-86	8	0.899	0.178	0.709	1.298

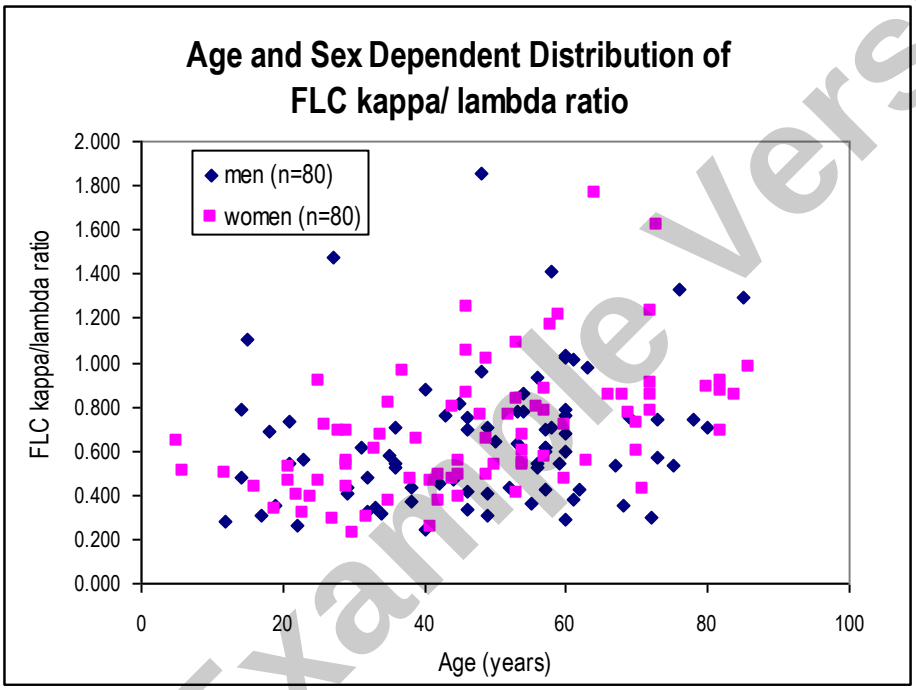

Figure 4c: FLC kappa/lambda ratio plotted against donor age.

16. METHOD COMPARISON

The BioVendor Human Immunoglobulin Free Light Chain Kappa and Lambda ELISA kit was compared to the other commercial immunoassay (nephelometric assay), measuring of 19 serum samples. The following correlation graphs were obtained:

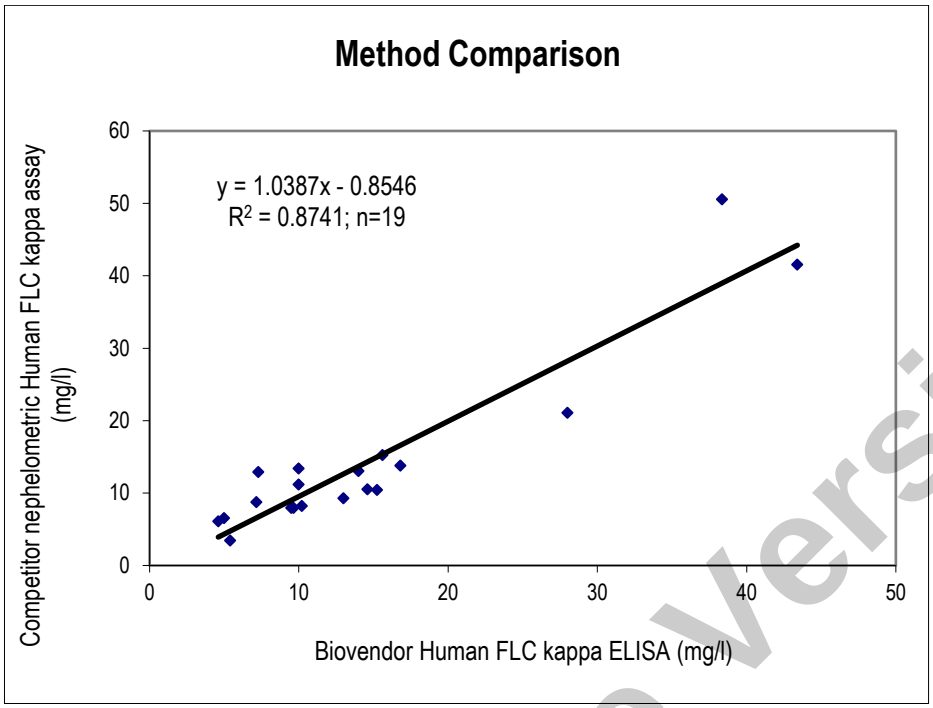

Figure 5a: Method comparison FLC kappa.

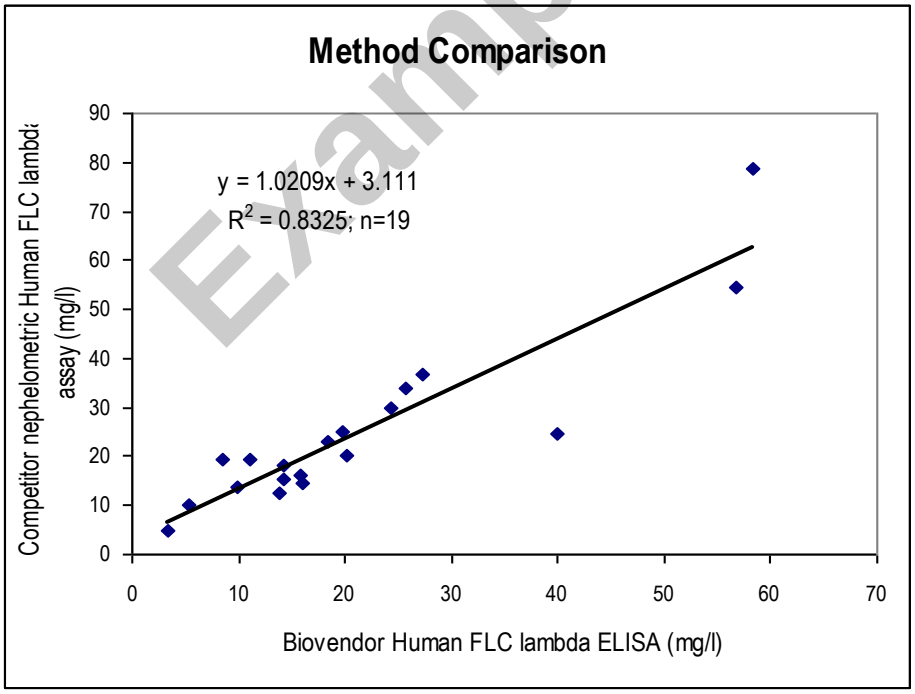

Figure 5b: Method comparison FLC lambda.

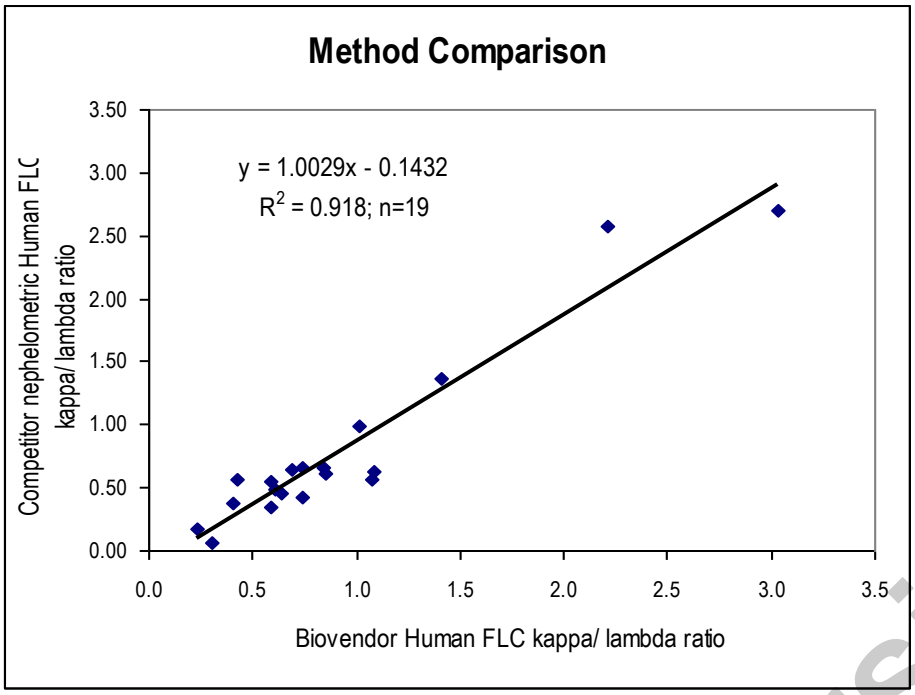

Figure 5c: Method comparison FLC kappa/lambda ratio.

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

References to FLCs:

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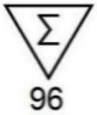
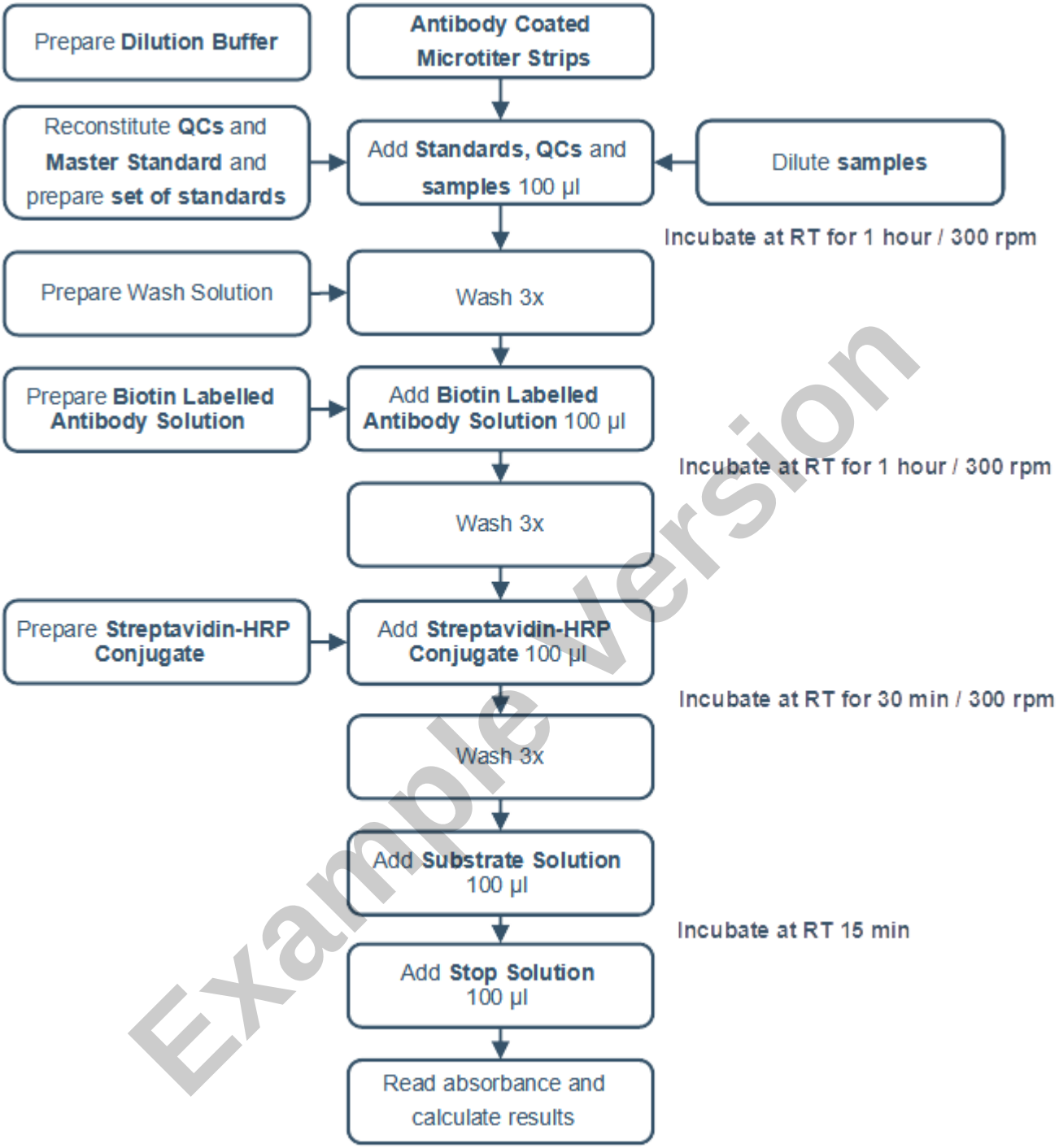
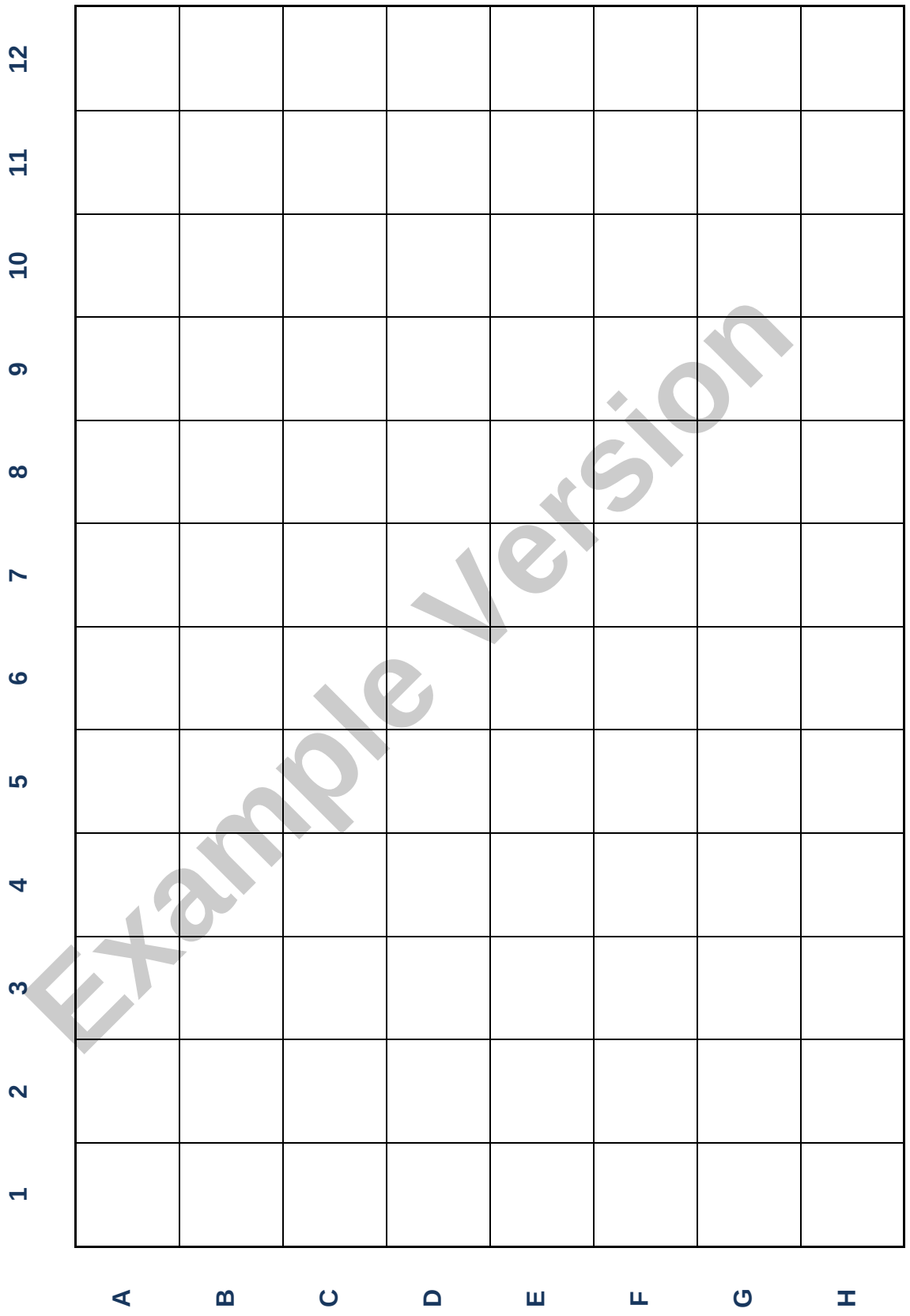
For more references on this product see our web pages at www.biovendor.com.

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

20. ASSAY PROCEDURE - SUMMARY





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