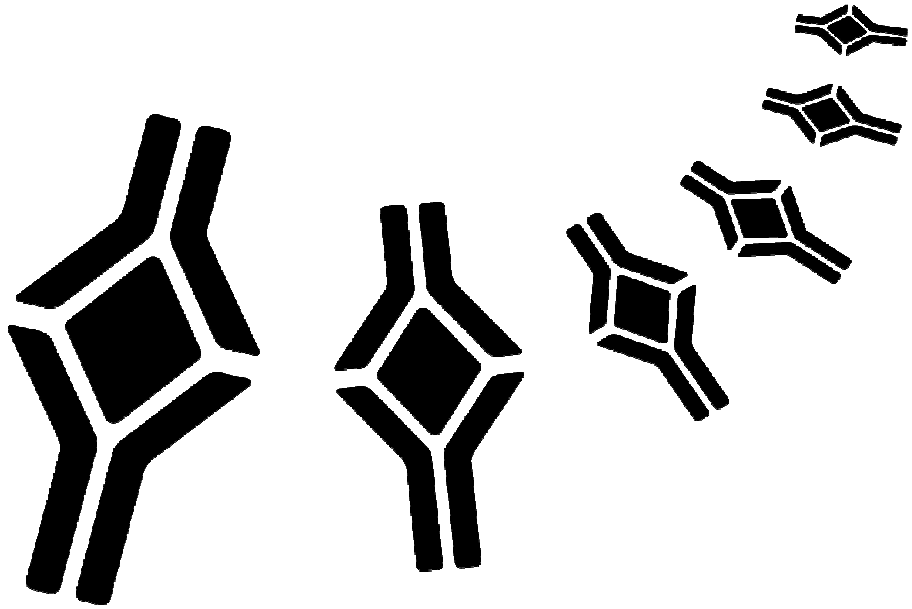
BioVendor
Research
and Diagnostic Products



HUMAN CLARA CELL PROTEIN ELISA

Product Data Sheet

Cat. No.: RD191022200

European Union: IVD CE

Rest of the world:
For research use only!

VERSION 03 150311 19

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 RD191022200 Human Clara Cell Protein ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Clara cell protein.

Features

- **European Union: for in vitro diagnostic use**
  Rest of the world: for research use only!
- The total assay time is less than 4 hours
- The kit measures total (homodimeric) Clara cell protein in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is purified native protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

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

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Human Clara cell protein (CC16, CC10 and also called uteroglobin, urinary protein 1 or Clara cell secretory protein) belongs to the family of secretoglobins and is a secreted protein product of non-ciliated bronchiolar Clara cells. Its function remains to be elucidated but there is convincing data suggesting its phospholipase A2 inhibitory activity as well as a number of other immunomodulatory features including inhibition of interferon gamma signaling and Th1 vs. Th2 lymphocyte regulation.
It was proposed as a potential peripheral marker of respiratory epithelial injury and bronchial dysfunction.
Clara cell protein 16 concentrations have been determined in both serum and bronchoalveolar lavage fluid in numerous studies since 1994. In serum, its increase is associated with age, asbestos, nitrogen chloride and ozone exposure, sarcoidosis and high PEEP ventilation. Decreased serum CC16 levels are found after pulmonary resection, in silica-exposed workers, smokers and in asthma.
Decreased CC16 concentrations were also found in the amniotic fluid of fetuses suffering from pulmonary hypoplasia caused by various mechanisms (diaphragmatic hernia, diabetic fetopathy, Turner and Down syndrome). In pleural effusions, the CC16 concentration appears to be associated with its diffusion from the lung as evidenced by high CC16 levels in cardiac pleural congestion.

Areas of investigation:
Lung inflammation and infection
Lung cancer
IgA-nephropathy

4. TEST PRINCIPLE

In the BioVendor Human Clara Cell Protein ELISA, standards, quality controls and samples are incubated in microplate wells pre-coated with polyclonal anti-human Clara cell antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-human Clara cell protein antibody is added and incubated with captured Clara cell protein for 60 minutes. After another washing, streptavidin-horseradish peroxidase conjugate is added. After 60 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 Clara cell protein. 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
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody	ready to use	13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	1 vial
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	20 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml
Product Data Sheet + Certificate of Analysis	-	1 pc

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precision pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

» **All reagents need to be brought to room temperature prior to use**

» **Always prepare only the appropriate quantity of reagents for your test**

» **Do not use components after the expiration date marked on their label**

- **Assay reagents supplied ready to use:**

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

Biotin Labelled Antibody
Streptavidin-HRP Conjugate
Dilution Buffer
Substrate Solution
Stop Solution
Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

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

Human Clara cell protein 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 Clara cell protein in the stock solution is **100 ng/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	-	100 ng/ml
200 µl of stock	300 µl	40 ng/ml
200 µl of 40 ng/ml	200 µl	20 ng/ml
200 µl of 20 ng/ml	200 µl	10 ng/ml
200 µl of 10 ng/ml	200 µl	5 ng/ml
200 µl of 5 ng/ml	300 µl	2 ng/ml

Dilute prepared standards (100 - 2 ng/ml) 25x with Dilution Buffer just prior to the assay, e.g. 10 µl of Standard + 240 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

Standard stock solution (100 ng/ml) should be aliquoted and frozen at –20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Standard solutions.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of distilled water needed for reconstitution and for current Quality Control concentration!!!

Reconstitute each Quality Control (HIGH and LOW) with distilled water just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).

Dilute reconstituted Quality Controls 25x with Dilution Bufffer, e.g. 5 µl of Quality Control + 120 µl of Dilution Buffer when assaying samples in singlets, or preferably 10 µl of Quality Control + 240 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:

The reconstituted Quality Controls must be used immediately or aliquoted and frozen at -20°C for 3 months. Avoid repeated freeze/thaw cycles.

Do not store the diluted Quality Controls.

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 Clara cell protein in serum and plasma (EDTA, citrate, heparin).

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

Dilute serum or plasma samples 25x with Dilution Buffer just prior to the assay, e.g. 5 µl of sample + 120 µl of Dilution Buffer for singlets, or preferably 10 µl of sample + 240 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

In case of measurement of Clara cell protein in bronchoalveolar lavage fluid an appropriate dilution should be assessed by the researcher in advance to batch measurement (recommended starting dilution is 750x).

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

See Chapter 13 for stability of serum and plasma samples when stored at 2-8°C, effect of freezing/thawing and effect of sample matrix (serum/plasma) on the concentration of Clara cell protein.

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 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 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 **1 hour**, 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 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: 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 Clara cell protein 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 100**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 40**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 20**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 10**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 5**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 2**	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 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 Clara cell protein 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.

Samples, Quality Controls and Standards are all diluted 25x prior to analysis, so there is no need to take this dilution factor into account.

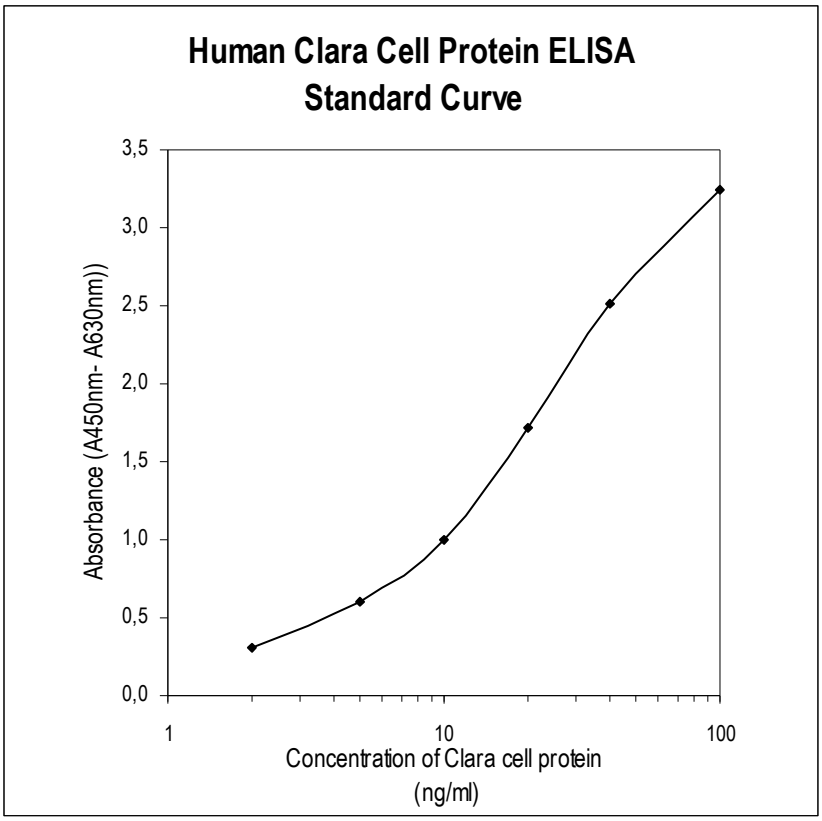


Figure 2: Typical Standard Curve for Human Clara Cell Protein ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human Clara Cell Protein 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 Clara cell protein values in wells and is 20 pg/ml.
*Dilution Buffer is pipetted into blank wells.

- **Limit of assay**

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

- **Specificity**

The antibodies used in this ELISA are specific for human Clara cell protein with no detectable crossreactivities to the cytokines that may be present in human serum.

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

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	5.09	0.29	5.7
2	5.75	0.30	5.18

Inter-assay (Run-to-Run) (n=4)

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	5.31	0.21	4.1
2	5.74	1.19	3.4
3	10.92	0.69	6.4

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Clara cell protein, diluted with Dilution Buffer 25x and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	13.21	-	-
	18.44	18.21	101.3
	23.20	23.21	100.0
	32.50	33.21	97.9
2	7.27	-	-
	12.14	12.27	98.9
	16.58	17.27	96.0
	25.12	27.27	92.1

- **Linearity**

Serum samples (diluted 25x with Dilution Buffer) were serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	16.98	-	-
	2x	8.69	8.49	102.4
	4x	5.02	4.25	118.3
	8x	2.33	2.12	109.8
2	-	10.66	-	-
	2x	5.48	5.33	102.8
	4x	2.86	2.67	107.3
	8x	1.24	1.33	93.1

- **Effect of sample matrix**

EDTA, citrate and heparin 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	19.0	17.8	16.0	22.1
2	8.3	8.4	7.0	8.0
3	6.9	5.9	5.7	6.0
4	10.4	9.6	9.1	10.0
5	5.1	5.3	4.5	4.6
6	22.0	20.4	17.9	26.0
7	14.0	13.8	11.9	12.7
8	7.6	7.6	6.3	8.9
9	11.9	11.7	10.0	12.1
10	13.2	15.4	11.9	13.7
Mean (ng/ml)	**11.84**	**11.59**	**10.03**	**12.41**
Mean Plasma/Serum (%)	**-**	**97.9**	**84.7**	**104.8**
Coefficient of determination R^2	**-**	**0.96**	**0.99**	**0.97**

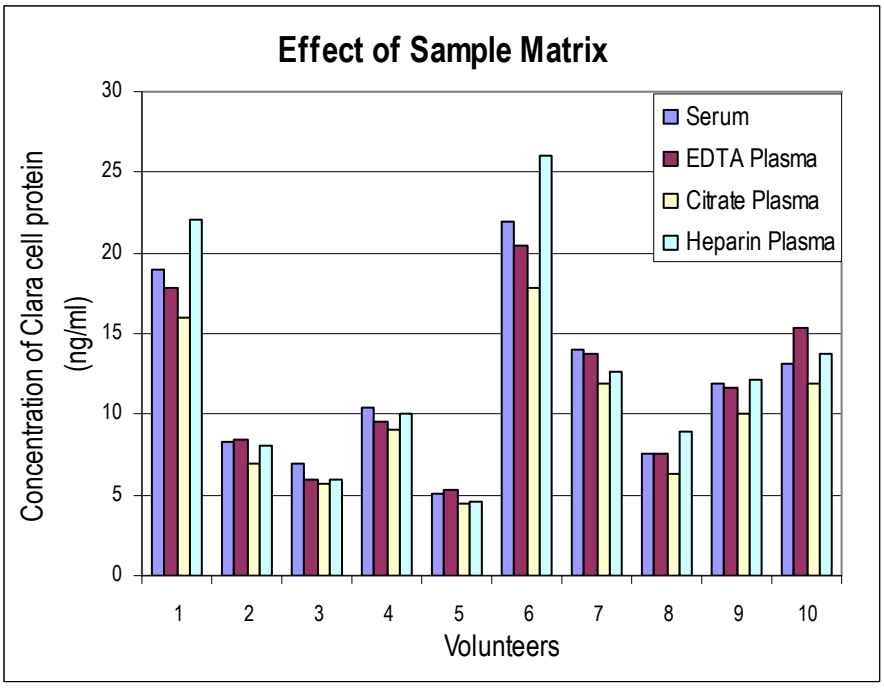


Figure 3: Human Clara cell protein levels measured using Human Clara Cell Protein ELISA from 10 individuals using serum, EDTA, citrate and heparin plasma, respectively.

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

Samples should be stored at –20°C. However, no decline in concentration of Clara cell protein was observed in serum and plasma samples after 14 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.

- **Effect of Freezing/Thawing**

No decline was observed in concentration of human Clara cell protein in serum and plasma samples after repeated (3x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

14. DEFINITION OF THE STANDARD

The native protein is used as the Master Standard in this assay. The native Clara cell protein is purified from human urine and the Clara cell protein is a 16 kDa dimeric protein consisting of two disulfide-linked polypeptide chains.

The CC16 concentration strongly depends on the method, which is used for the protein determination. Master Standard contains 50 ng of CC16 measured by Bradford method (used in this kit), 215 ng of CC16 measured by BCA method and 340 ng of CC16 measured by Lowry method.

15. PRELIMINARY POPULATION AND CLINICAL DATA

- **Typical distribution of Clara cell protein in various body fluids**

Sample	*Mean (ng/ml)*	*Range (ng/ml)*
Serum	12.6	3.7 - 23.2
Urine	18.7	0.2 - 88.6
Seminal fluid	1 030.0	145 – 8 600
BAL	1 360.0	154 – 4 300
Synovial fluid	9.1	2.8 - 16.4
Pleural fluid	11.4	0.7 - 32.8
Cerebrospinal fluid	0.5	0 - 5.7
Gastric juice	185.0	0 – 1 220
Bile	0.7	0 - 2.3

Concentrations of Clara cell protein are expressed as ng/ml. See for details:

- Shijubo N., Kawabata I., Sato N., Itoh Y.: Clinical Aspects of Clara Cell 10-kDa Protein/ Uteroglobin (Secretoglobin 1A1), Current Pharmaceutical Design, 9, 1139-1149, (2003)

- **Reference range**

It is recommended that each laboratory include its own panel of control sample in the assay. Each laboratory should establish its own normal and pathological reference ranges for Clara cell protein levels with the assay.

16. METHOD COMPARISON

The BioVendor's Human Clara Cell Protein ELISA was compared to a latex imunoassay. Linear regression analysis of the results yielded the following results.

ELISA = 0.86 x LATEX $r^2 = 0.86$

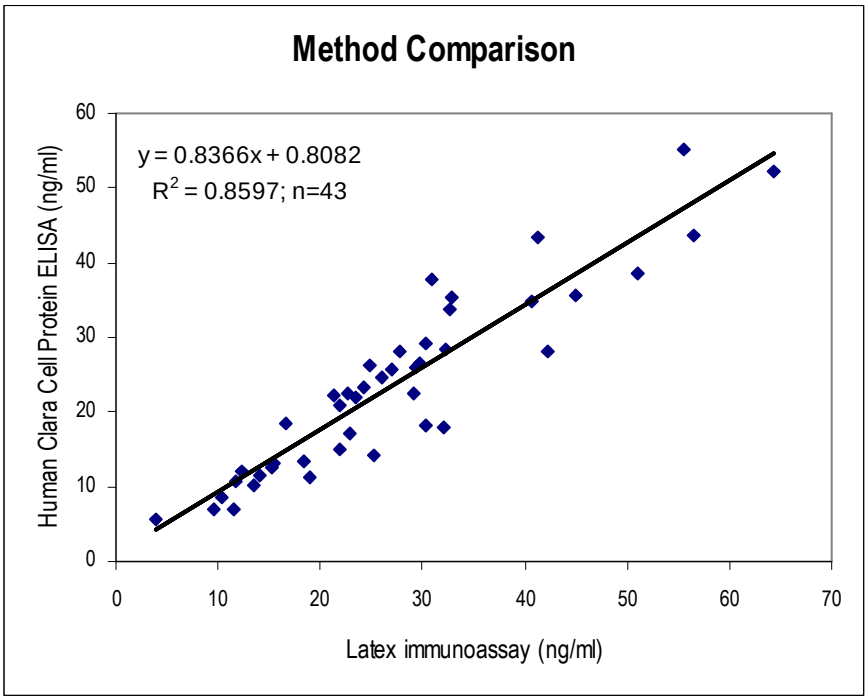


Figure 4: Method comparison.

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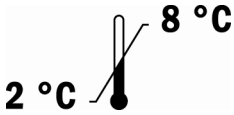
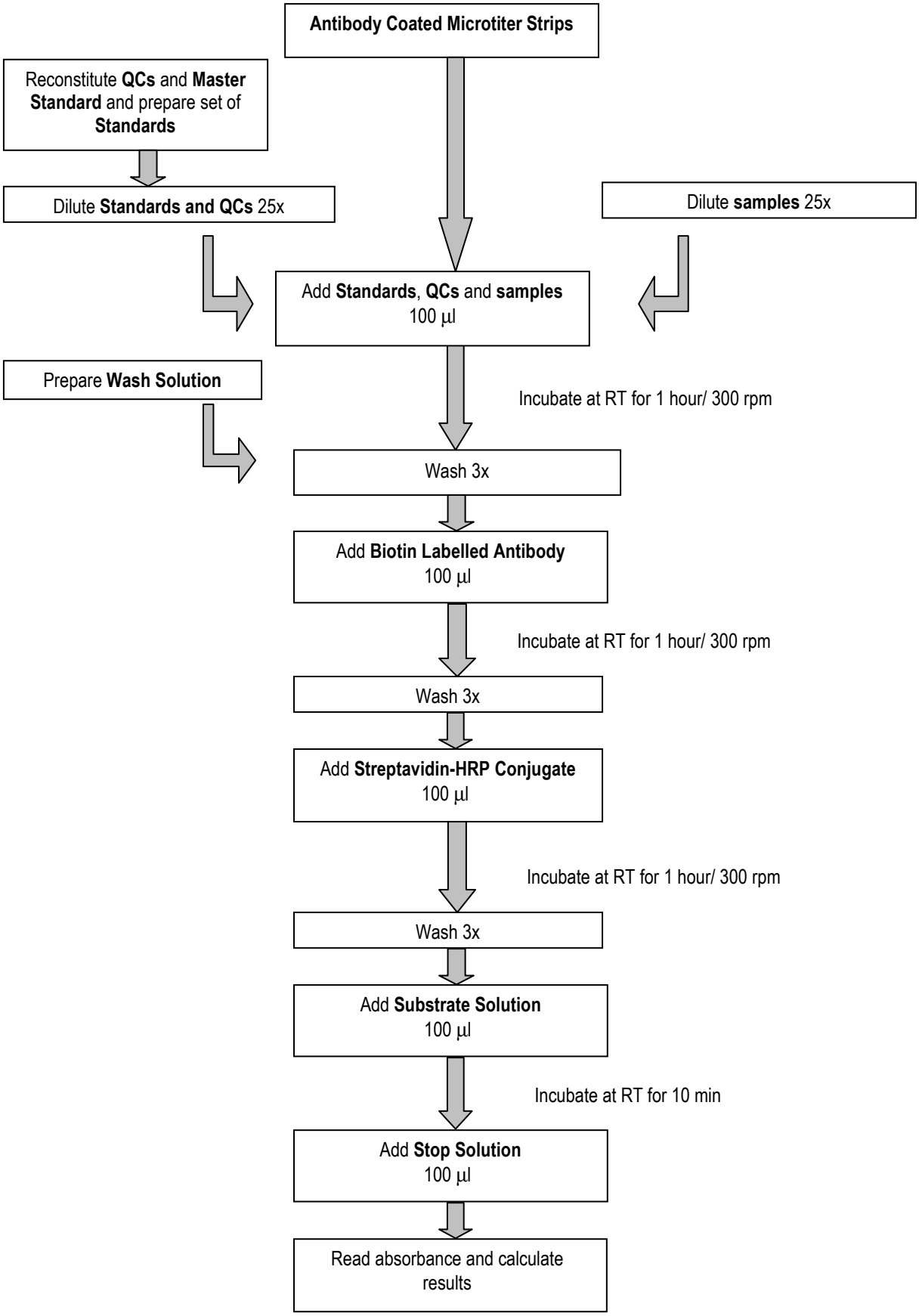
» For more references on this product see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
	Storage conditions
	Identification of packaging materials
IVD CE	In vitro diagnostic medical device

Assay Procedure Summary



	1	2	3	4	5	6	7	8	9	10	11	12
A												
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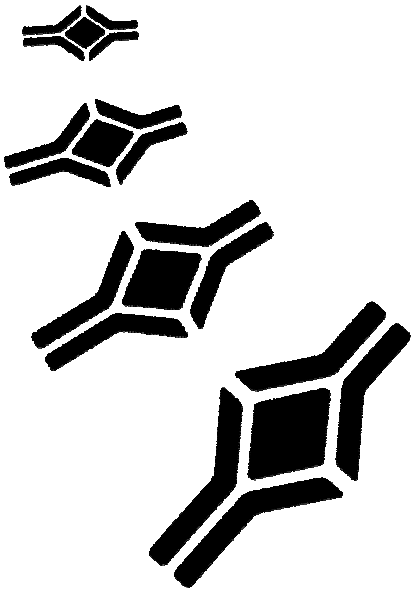

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