Human Clara Cell Protein ELISA

Cat. No.: RD191022200R

Manufacturer

BioVendor - Laboratorní medicína a.s.

Place of business:
CTPark Modrice
Evropska 873
664 42 Modrice
CZECH REPUBLIC
e-mail: info@biovendor.com
http://www.biovendor.com
Phone: +420-549 124 185
Fax: +420-549 211 460

Registered office:
Tumova 2265/60
616 00 Brno
CZECH REPUBLIC

Daughter companies

European Union:

BioVendor GmbH
Im Neuenheimer Feld 583
D-69120 Heidelberg, GERMANY
Phone: +49-6221-433-9100
Fax: +49-6221-433-9111
e-mail: roth@biovendor.com
e-mail: abels@biovendor.com

USA, Canada and Mexico:

BioVendor, LLC
1463 Sand Hill Road, Suite 227
Candler, NC 28715, USA
Phone: 828-670-7807, 800-404-7807
Fax: 828-670-7809
e-mail: slee@biovendor.com
e-mail: worden@biovendor.com

China - Hong Kong Office:

BioVendor Laboratories Ltd.
Room 4008
Hong Kong Plaza, No.188
Connaught Road West
Hong Kong, CHINA
Phone: +852 28030523
Fax: +852 28030525
e-mail: lu@biovendor.com

China – Mainland Office:

BioVendor Laboratories Ltd.
Room 2405
YiYa Tower, TianYu Garden, No.150
Lihe Zhong Road
Guang Zhou, CHINA
Phone: +86-20-38840399, 38840386
Fax: +86-20-38840386
e-mail: jhsu@biovendor.com

CONTENTS:

Use only the actual version of Product Data Sheet enclosed with the kit!

1. Intended Use

The RD191022200R Human Clara Cell Protein ELISA is a biotin labelled antibody based sandwich enzyme immunoassay for the quantitative measurement of human Clara Cell Protein in serum, plasma, tissue culture medium and bronchoalveolar lavage fluid. It is intended for *in vitro* research use only.

Features

- The total assay time is less than four hours.
- The kit measures total (homodimeric) Clara Cell Protein.
- Quality Controls are human serum based. No animal sera are used.

2. Storage, Expiration

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

3. Summary

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.

4. Test Principle

In the BioVendor's Human Clara Cell Protein ELISA, calibrators or samples are incubated with a rabbit polyclonal anti-human Clara Cell Protein antibody coated in microtiter wells. After one-hour incubation and a washing, biotin-labelled polyclonal anti-human Clara Cell Protein antibody is added and incubated with captured Clara Cell Protein. After a thorough wash, streptavidin-horseradish peroxidase conjugate is added. After one hour incubation and the last washing step, the remaining conjugate is allowed to react with the substrate H_2O_2-tetramethylbenzidine. The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured at 450 nm. The absorbance is proportional to the concentration of Clara Cell Protein. A standard curve is constructed by plotting absorbance values versus Clara Cell Protein concentrations of calibrators, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- For *in vitro* and research use only.
- This kit contains components of human origin. These materials were found non-reactive for HbsAg, HCV antibody and for HIV 1/2 antibody and antigen. However, these materials should be handled as potentially infectious, as no tests can guarantee the complete absence of infectious agents.
- Avoid contact with the acidic Stop Solution and Substrate Solution which contains hydrogen peroxide. Wear gloves and eye protection when handling these reagents. In case of contact with the Stop Solution and the Substrate Solution wash skin thoroughly with water and seek medical attention, when necessary.
- Wear gloves and laboratory coats when handling immunodiagnostic materials.
- The materials must not be pipetted by mouth.
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled.
- Reagents with different lot numbers should not be mixed.
- Reagents should not be used beyond the expiration marked on kit label.

6. Reagents Supplied

Cat. No.	*Kit Components*	*Quantity*
C071111	Antibody Coated Microtiter Strips, sealed	96 wells
C072511	Biotin Labelled Antibody, ready to use	13 ml
C072351	Streptavidin-HRP Conjugate, ready to use	13 ml
C073141	Clara Cell Protein Master Calibrator, lyophilized	1 vial
C074191	Quality Control High, lyophilized	2 vials
C074251	Quality Control Low, lyophilized	2 vials
C005114	Dilution Buffer, ready to use	20 ml
C006121	Wash Solution Concentrate (10x)	100 ml
C007111	Substrate Solution (TMB), ready to use	13 ml
C008111	Stop Solution (0.2 M H_2SO_4), ready to use	13 ml
-	Instruction Manual + Certificate of Analysis	1 pc

7. Materials Required but Not Supplied

- Test tubes for diluting samples
- Precision pipettes to deliver 10-1000 µl and disposable tips
- Multichannel pipette 100 µl
- Glassware (graduated cylinder and bottle for Wash Solution)
- Deionized (distilled) water
- Microtitration plate washer (optional) [Manual washing is possible but not preferable.]
- Microplate reader with 450 nm filter
- Orbital microplate shaker capable of approximately 300 rpm
- Software package facilitating data generation and analysis (optional)

8. Preparation of Reagents

All reagents need to be brought to room temperature prior to the assay.

Assay reagents are supplied ready to use, with the exception of Clara Cell Protein Master Calibrator, Quality Controls and Wash Solution Concentrate (10x).

Preparation of reagents for 1 plate:

- If you do not use the whole plate, return unused strips in the provided aluminium bag with desiccant and seal the bag carefully. Keep the unused strips at 2-8°C, protected from the moisture.

Wash Solution:
Dilute 100 ml of Wash Solution concentrate with 900 ml of deionized (distilled) water.

Stability and storage:
The diluted Wash Solution is stable for 1 month when stored at 2-8°C.

Human Clara Cell Protein Calibrator:
Reconstitute Clara Cell Protein Master Calibrator with 0.5 ml of Dilution Buffer, let it dissolve at least 15 minutes and shake gently (not to foam). The concentration of the human Clara Cell Protein in the stock solution is 100 ng/ml.
Prepare Calibrators as follows:

Calibrator volume	*Dilution Buffer volume*	*Concentration*
stock	-----	100 ng/ml
200µl of stock	300 µl	40 ng/ml
200 µl of std.40	200 µl	20 ng/ml
200 µl of std. 20	200 µl	10 ng/ml
200 µl of std. 10	200 µl	5 ng/ml
200 µl of std. 5	300 µl	2 ng/ml

Dilute prepared Calibrators 1:25 with Dilution Buffer prior to use in ELISA, e.g. 10 µl calibrator solution + 240 µl Dilution Buffer for duplicates. Do not store the diluted Calibrators.

Stability and storage:
Reconstituted and undiluted Calibrators should be frozen at -20°C until next use.

Do not store the diluted (25x) Calibrators.

Quality Controls:
Dissolve lyophilized Quality Controls with 0.1 ml of distilled water, let stand at least 15 minutes and shake gently (not to foam). These solutions are prepared for subsequent diluting.
Dilute Quality Controls prior to use 1:25 with Dilution Bufffer, e.g. 5 ul sample + 120 ul Dilution Buffer when assaying samples in singlets, or preferably 10 ul sample + 240 ul Dilution Buffer for duplicates.

Stability and storage:
Reconstituted but undiluted Quality Controls are stable up to one month when stored at –20°C.
Do not store the diluted (1:25) Quality Controls

9. Preparation of Samples

Dilute serum or plasma samples 1:25 with Dilution Buffer, e.g. 5 µl sample + 120 µl Dilution Buffer when assaying samples in singlets, or preferably 10 µl sample + 240 µl Dilution Buffer for duplicates.
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 1:750).
Do not store the diluted samples.

Stability and storage:
Serum and plasma samples are stable for 1 year when stored at –20°C and for 2 years when stored at –70°C.

10. Assay Procedure

1) Pipet 100 µl of diluted Calibrators, 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).
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).
7) Add 100 µl of Streptavidin-HRP Conjugate.
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).
10) Add 100 µl of Substrate Solution. (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.). No shaking!
12) Stop the colour development by adding 100 µl of Stop Solution.
13) Determine the absorbance by reading the plate at 450 nm. (The absorbance should be read within 5-15 minutes following step 12.)

Note: If the microplate reader is not capable of reading absorbance greater than the absorbance of the highest standard, 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 samples. The readings at 405 nm should not replace the on-scale readings at 450 nm.

.

Figure 1: Example of work sheet.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	Calibrator 100	QC Low	Sample 8	Sample 16	Sample 24	Sample 32
B	Calibrator 40	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	Calibrator 20	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	Calibrator 10	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	Calibrator 5	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	Calibrator 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

11. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of calibrators versus *log* of the known concentration (X) of calibrators, using the four-parameter function.

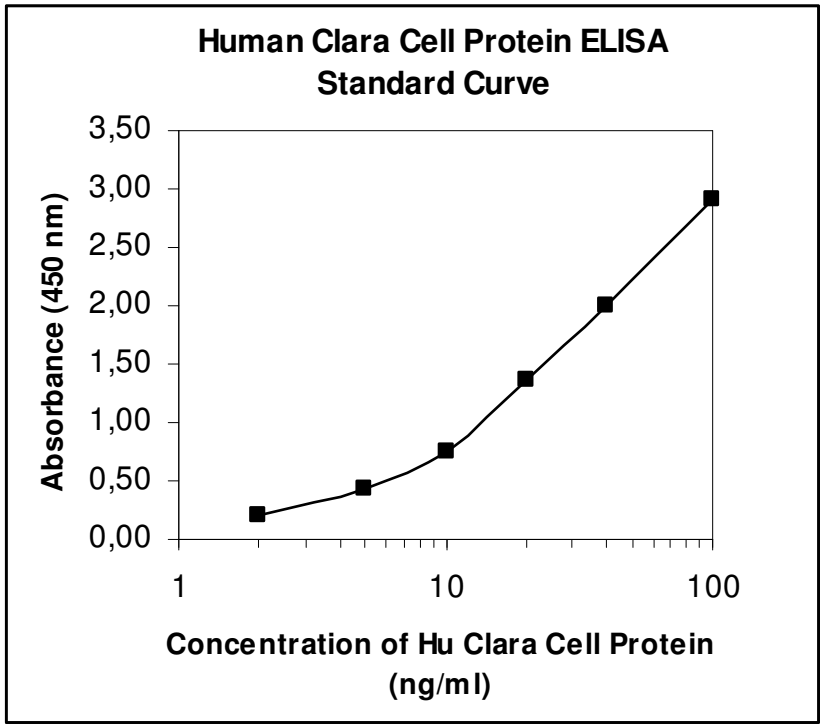


Figure 2: Standard Curve for Clara Cell Protein is plotted using the four-parameter function as a proportion of Clara Cell Protein concentration and absorbance at 450 nm.

Alternatively, the *logit log* function can be used to linearize the calibration curve (i.e. *logit* of absorbance (Y) is plotted versus *log* of the known concentration (X) of calibrators).

Serum or plasma samples, Quality Controls and Calibrators are diluted 1:25 prior to analysis, so there is no need to account for this dilution.

12. Limits of Assay

Results exceeding Clara Cell Protein level of 100 ng/ml should be repeated with diluted samples. Dilution factors need to be taken into consideration in calculating the Clara Cell Protein concentration.

13. Performance Characteristics

Typical analytical data obtained with the BioVendor Human Clara Cell Protein ELISA are presented in this chapter. For actual Standard curve and Quality Controls values see the Certificate of Analysis.

- **Sensitivity**

The limit of detection (defined as human Clara Cell Protein concentration giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is defined as follows:
Analytical Limit of Detection is calculated from the real human Clara Cell Protein values in wells and is 20 pg/ml
Assay Sensitivity takes the dilution of samples into consideration and is calculated according to the formula:
Assay Sensitivity = Analytical Limit of Detection x sample dilution = 20 pg/ml x 25 = 500 pg/ml

*Dilution Buffer is pipetted into blank wells.

- **Specificity**

The antibodies in the Human Clara Cell Protein ELISA kit are highly specific for human Clara Cell Protein with no detectable crossreactivities to the cytokines that may be present in human serum.

- **Precision**

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	4.52	0.33	7.3
2	7.96	0.64	8.1
3	15.54	1.23	7.9
4	19.44	0.93	4.8

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

Sample	*Mean (ng/ml)*	*SD (ng/ml)*	*CV (%)*
1	3.93	0.43	10.9
2	5.98	0.16	2.6
3	6.94	0.61	8.7
4	10.58	0.17	1.6

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Clara Cell Protein, diluted with Dilution Buffer 1:25 and assayed.

Sample	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	6.69	-	-
	11.29	11.41	98.9
	17.03	16.32	104.4
	25.22	24.22	104.1
2	13.53	-	-
	20.26	18.25	111.0
	26.04	23.16	112.4
	33.40	31.06	107.5

- **Linearity**

Serum samples (diluted 1:25 with Dilution Buffer) were further serially diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (ng/ml)*	***E**xpected (ng/ml)*	*Recovery **O/E** (%)*
1	-	18.18	-	-
	1:2	9.42	9.09	103.6
	1:4	4.50	4.55	99.0
	1:8	2.20	2.27	96.8
2	-	15.24	-	-
	1:2	8.10	7.62	106.3
	1:4	3.99	3.81	104.7
	1:8	1.84	1.91	96.6

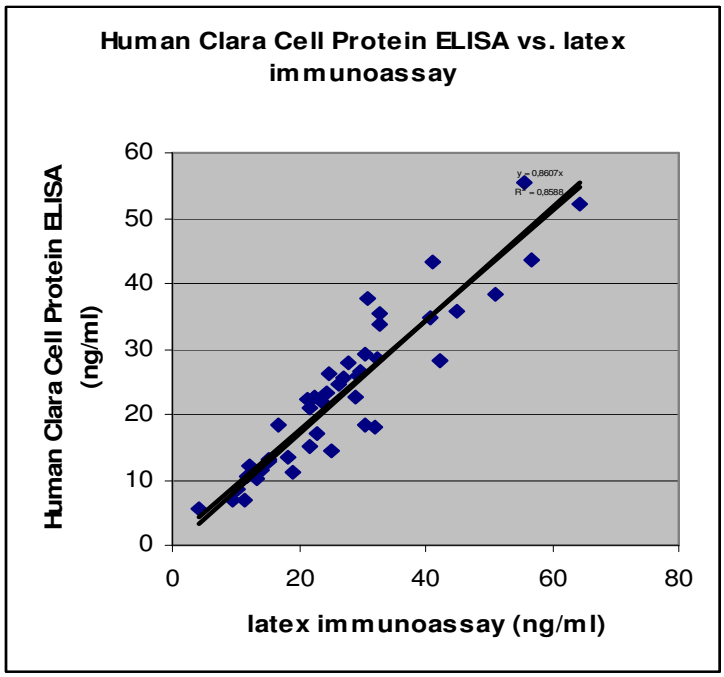
14. Definition of Clara Cell Protein Master Calibrator

The Clara Cell Protein is purified from human urine and is used as the calibrator. 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. 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 $r^2 = 0.86$



16. Troubleshooting and FAQs

1/ 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

2/ High signal and background in all wells
Possible explanations:
· Improper or inadequate washing
· Overdeveloping; incubation time should be decreased before addition of Stop Solution

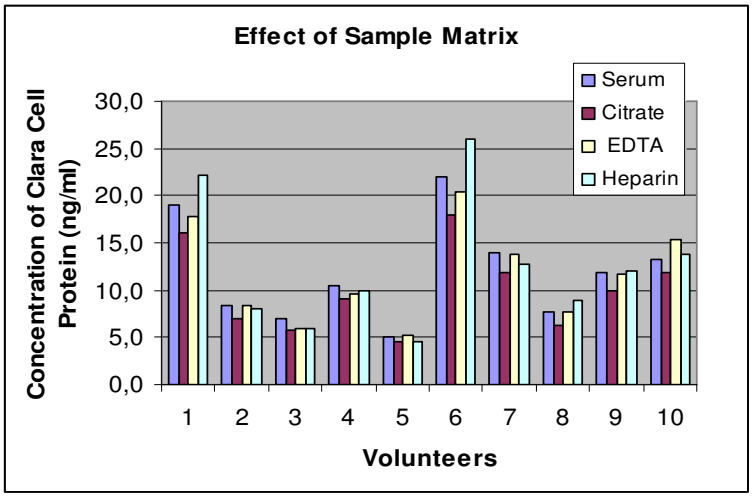
3/ High coefficient of variation (CV)
Possible explanation:
· Improper or inadequate washing

4/ Effect of freezing/thawing on the concentration of Clara Cell Protein in samples
No decline was observed in concentration of Clara Cell Protein in serum and plasma samples after repeated (3x) freezing/thawing cycles. Avoid repeated freezing/thawing of BAL samples.

5/ Effect of sample matrix (serum/plasma)

Samples from 10 healthy individuals were taken and treated by different methods, results shown below:

Volunteer	Serum (ng/ml)	Plasma (ng/ml)		
		Citrate	EDTA	Heparin
1	19.0	16.0	17.8	22.1
2	8.3	7.0	8.4	8.0
3	6.9	5.7	5.9	6.0
4	10.4	9.1	9.6	10.0
5	5.1	4.5	5.3	4.6
6	22.0	17.9	20.4	26.0
7	14.0	11.9	13.8	12.7
8	7.6	6.3	7.6	8.9
9	11.9	10.0	11.7	12.1
10	13.2	11.9	15.4	13.7



Mean values of Clara Cell Protein in serum, citrate plasma, EDTA plasma and heparin plasma, expressed in ng/ml:

Sample (n=10)	*Mean (ng/ml)*	*Plasma/Serum (%)*
Serum	11.0	-
Citrate Plasma	10.0	85
EDTA Plasma	11.6	97
Heparin Plasma	12.4	105

6/ Stability of samples at 4°C

Samples should be stored at –20°C. However, no decline was observed in concentration of Clara Cell Protein in serum and plasma samples when stored at 4°C for 2 weeks. To avoid microbial contamination, add NaN_3 to a final concentration 0.1% to the samples.

7/ Why are calibrator solutions diluted 25x prior to use in ELISA and what does it mean for calculation of results?

The dilution buffer suppresses the matrix effect of samples. It is a common practice to use the same dilution for samples and calibrators because of simple calculation of results: concentrations of samples can be read directly off the curve without considering a dilution factor. Thus, in Human Clara Cell Protein ELISA the same dilution of 1:25 is used for calibrators, controls and serum/plasma samples.

Samples exceeding Clara Cell Protein level of 100 ng/ml should be measured at higher degree of dilution (e.g. BAL samles at 1:750) and dilution factors need to be taken into consideration when calculating Clara Cell Protein concentrations then (the dilution factor is 30 in this case).

8/ Typical distribution of Clara Cell Protein in various body fluids

	Mean (ng/ml)	*Range (ng/ml)*
Serum	12.6	3.7-23.2
Urine	18.7	0.2-88.6
Seminal fluid	1030.0	145-8600
BAL	1360.0	154-4300
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	0-1220
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)

9/ A Need for more of Dilution Buffer

Dilute Dilution Buffer 1:1 with 0,9% NaCl und use this mixture for dilution of calibrator solutions and samples. The mixture is full compatible with original Dilution Buffer.

17. References

References to Clara Cell Protein

- Backé E, Lotz G, Tittelbach U, Plitzko S, Gierke E and Schneider WD: Immunological biomarkers in salt miners exposed to salt dust, diesel exhaust and nitrogen oxides. *Int Arch Occup Environ Health*, Jun; 77(5):319-27 (2004)
- Signor L, Tigani B, Beckmann N, Falchetto R and Stoeckli M: Two-dimensional electrophoresis protein profiling and identification in rat bronchoalveolar lavage fluid following allergen and endotoxin challenge. Proteomics, Jul; 4(7):2101-10 (2004)
- 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)
- Nord M., Schubert K., Cassel T., Andersson O., Riise G.: Decreased serum and bronchoalveolar lavage levels of Clara cell secretory protein (CC16) is associated with bronchiolitis obliterans syndrome and airway neutrophilia in lung transplant recipients. Transplantation, 73, 1264-1269, (2002)
- Petrek M., Hermans C., Kolek V., Fialova J., Bernard A.: Clara cell protein (CC16) in serum and bronchoalveolar lavage fluid of subjects exposed to asbestos. Biomarkers, 7(1), 58-67, (2002)
- Robin M., Dong P., Hermans C., Bernard A., Bersten A.D., Doyle I.R.: Serum levels of CC16, SP-A and SP-B reflect tobacco-smoke exposure in asymptomatic subjects. Eur Respir J, 20(5), 1152-1162, (2002)
- Hermans C., Petrek M., Kolek V., Weynand B., Pieters T., Lambert M., Bernard A.: Serum Clara cell protein (CC16), a marker of the integrity of the air-blood barrier in sarcoidosis. Eur Respir J, 18(3), 507-514 (2001)
- Linnoila I., Szabo E., deMayo F., Witschi H., Sabourin C., Malkinson A.: The role of CC10 in pulmonary carcionogenesis: from a marker to tumor suppression. Annals of the New York Academy of Science, 923, 249-267, (2000)
- Broeckaert F., Clippe A., Knoops B., Hermans C. and Bernard A.: Clara cell secretory protein (CC16): features as a peripheral lung biomarker. Annals of the New York Academy of Science, 923, 68-77, (2000)
- Shijubo N., Itoh Y., Yamaguchi T., Abe S.: Development of an enzyme-linked immunosorbent assay for Clara cell 10-kDa protein: in pursuit of clinical significance of sera in patients with asthma and sarcoidosis. Annals of the New York Academy of Science, 923, 268-279, (2000)
- Bernard A., Roels H., Lauwerys R., Witters R., Gielens C., Soumillion A. et al.: Human urinary protein 1: Evidence for identity with the Clara cell protein and occurrence in respiratory tract and urogenital secretions. Clin Chim Acta, 207, 239-249, (1992)

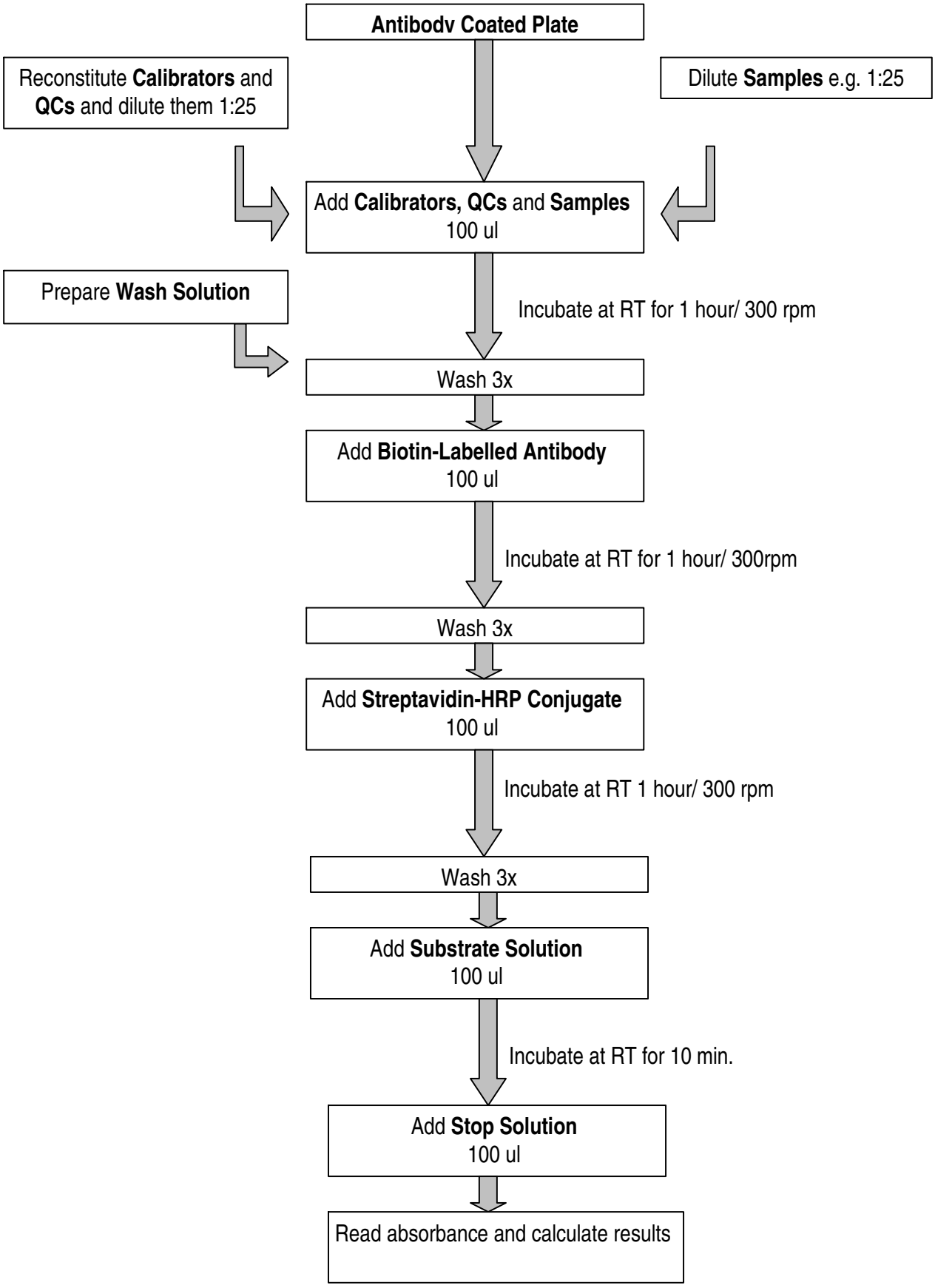
- Bernard A., Lauwerys R., Noel A., Vandeleene B., Lambert A.: Urine protein 1: a sex-dependent marker of tubular or glomerular dysfunction. Clin Chem, 35, 2141-2142, (1989)
- Jackson P.J., Turner R., Keen J.N., Brooksbank R.A., Cooper E.H.: Purification and partial amino acid sequence of human urine protein 1. Evidence for homology with rabbit uteroglobin. J Chromatography, 452, 359-367, (1988)

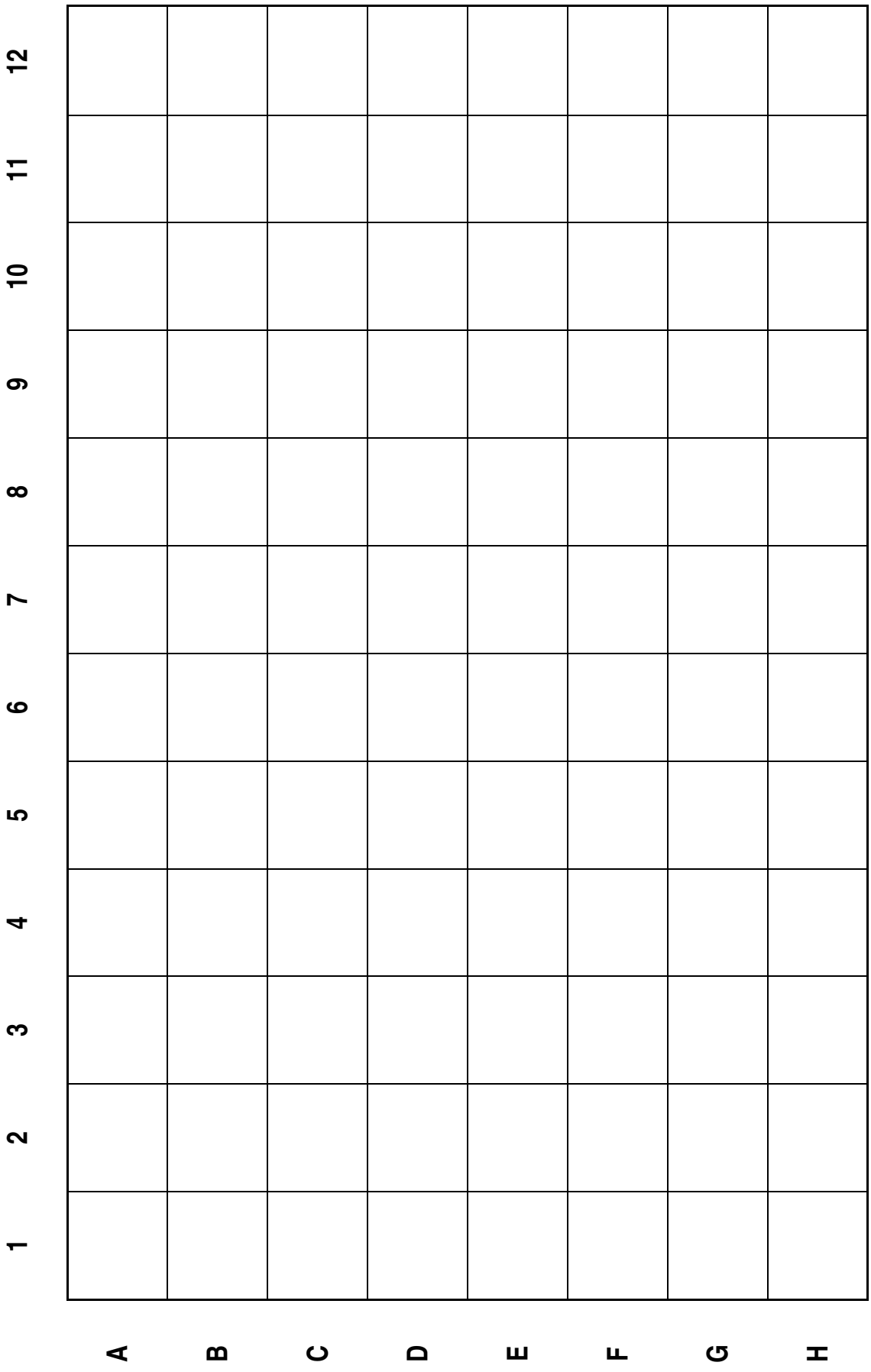
References to this product

- Benson M, Jansson L, Adner M, Luts A, Uddman R, Cardell LO: Gene profiling reveals decreased expression of uteroglobin and other anti-inflammatory genes in nasal fluid cells from patients with intermittent allergic rhinitis. Clin Exp Allergy, 35(4):473-84, (2005)
- Mattsson J, Remberger M, Andersson O, Sundberg B, Nord M: Decreased serum levels of clara cell secretory protein (CC16) are associated with bronchiolitis obliterans and may permit early diagnosis in patients after allogeneic stem-cell transplantation. Transplantation, 79(10):1411-6 (2005)

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

Assay Procedure Summary




12
11
10
9
8
7
6
5
4
3
2
1
A
B
C
D
E
F
G
H

Notes: