Human Clusterin ELISA

Cat. No.: RD194034200R

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

1. Intended Use

The RD194034200R Human Clusterin ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Clusterin.

Features

- It is intended for research use only.
- The total assay time is less than 3.5 hours.
- The kit measures Clusterin in serum, plasma, cerebrospinal fluid (CSF), urine, cell culture, and cell lysate.
- Components of the kit are provided ready to use, concentrated or lyophilized.
- Standard is recombinant protein based.
- Quality Controls are human serum based. No animal sera are used.

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 reagents see Chapter 9.

3. Introduction

Clusterin (Apolippoprotein J; SP-40,40; TRPM-2; SGP-2; pADHC-9; CLJ; T64; GP III; XIP8) is a highly conserved disulfide-linked secreted heterodimeric glycoprotein of 75-80 kDa but truncated forms targeted to nucleus have also been identified.
The protein is constitutively secreted by a number of cell types including epithelial and neuronal cells and is a major protein in physiological fluids including plasma, milk, urine, cerebrospinal fluid and semen. Due to its wide breath of tissue distribution many diverse physiological functions have been attributed to Clusterin including sperm maturation, membrane recycling, lipid transportation, tissue remodelling, complement inhibition and cell-cell or cell-substratum interactions. Moreover, it was propose, that Clusterin functions is as an extra cellular chaperon that stabilizes stressed proteins in a folding-competent state and protein has also been implicated in programmed cell death. Another defining prominent of Clusterin is its induction in many severe physiological disturbances states including kidney degenerative diseases, prostate and vesicle carcinogenesis, ovarian cancer and several neurodegenerative conditions (Alzheimer's disease).
Recent study demonstrate, that serum Clusterin level increases significantly in diabetic type II patients and in patients with developing coronary heart disease, or myocardial infarction. These date raise the possibility that elevated Clusterin levels in serum may represent a strong indication of vascular damage.
In patients with systemic lupus erythematosus (SLE) was found reduced serum Clusterin levels that correlated inversely with disease activity. Lowered Clusterin levels could be involved in the pathogeneses of SLE on account of decreased protective effects.
Another interesting observations obtain in rat model suggest that measurement of urinary Clusterin levels may be a useful clinical valuable marker for the severity of renal tubular damage. Furthermore, urinary Clusterin may also help to differentiate between tubular and glomerular forms of proteinuria.

Areas of investigation:
Coronary heart diseases
Myocardial infarction
Neurodegenerative diseases
Kidney degenerative disease
Renal tubular damage

4. Test Principle

In the Biovendor Human Clusterin ELISA, Standards, Quality Controls and diluted samples are incubated in microtitration wells pre-coated with monoclonal anti-human Clusterin antibody. After 60 minutes and washing, biotin labelled second monoclonal anti-human Clusterin antibody is added and incubated with the captured Clusterin 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 (0.2M H_2SO_4) and absorbance of the resulting yellow product is measured spectrophotometrically at 450 nm. The absorbance is proportional to the concentration of Clusterin. A standard curve is constructed by plotting absorbance values versus Clusterin concentrations of Standards, and concentrations of unknown samples are determined using this standard curve.

5. Precautions

- 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. Reagents Supplied

Kit Components	*State*	*Quantity*
Antibody Coated Microtiter Strips	sealed	96 wells
Biotin Labelled Antibody Concentrate (50x)	concentrated	0.25 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Human Clusterin Master Standard	lyophilized	2 vials
Quality Control High	lyophilized	2 vials
Quality Control Low	lyophilized	2 vials
Biotin-Ab Diluent	ready to use	13 ml
Dilution Buffer	ready to use	50 ml
Wash Solution Concentrate (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. Materials Required but Not Supplied

- Deionized (distilled) water
- Test tubes for samples dilution
- Glassware (graduated cylinder and bottle) for Wash Solution
- Absorbent material (e.g. paper towels) for blotting the microtiter plate after washing
- Vortex mixer
- Precision pipettes to deliver 5-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- 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
- 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-lock bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

- **Dilution Buffer**
- **Biotin-Ab Diluent**
- **Straptavidin-HRP Conjugate**
- **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 Clusterin Standards**:

Reconstitute the lyophilized Master Standard with 0.6 ml of 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 Clusterin in the stock solution is 160 ng/ml.
Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	*Dilution Buffer*	*Concentration*
Stock	------	160 ng/ml
300 µl of stock	300 µl	80 ng/ml
300 µl of "80 ng/ml"	300 µl	40 ng/ml
300 µl of "40 ng/ml"	300 µl	20 ng/ml
300 µl of "20 ng/ml"	300 µl	10 ng/ml
300 µl of "10 ng/ml"	300 µl	5 ng/ml

Stability and storage:
Do not store standard stock solution and other standards.

- **Quality Controls**:

Reconstitute each Quality Control (High and Low) with 0.5 ml of Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam). Quality controls are ready to use, do not dilute them.

Refer to the Certificate of Analysis for actual Quality Controls values.

Stability and storage:

Do not store reconstituted Quality Controls.

- **Wash Solution:**

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.

- **Biotin Labelled Antibody Solution**:

Prior to the assay prepare the working Biotin Labelled Antibody Solution by adding 1 part Biotin Labelled Antibody Concentrate (50x) with 49 parts Biotin-Ab Diluent. Prepare only the volume needed for the test. Mix thoroughly and gently.

Stability and storage:

Do not store diluted Biotin Labelled Antibody Solution.

Opened Biotin Labelled Antibody Concentrate (50x) is stable 3 months when stored at 2-8°C.

10. Preparation of Samples

The kit measures Human Clusterin in serum, plasma, CSF, urine, cell culture and cell lysate.

Samples should be assayed immediately after collection or should be stored at -20°C or -70°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.

Preparation of serum or plasma samples:
Dilute samples just prior to perform the test 3000-fold with Dilution Buffer in two steps as follows:
Dilution A (50-fold):
Add 5 µl of sample into 245 µl of Dilution Buffer and **mix well** (not to foam). Vortex is recommended.
Dilution B (60-fold):
Add 5 µl of Dilution A into 295 µl of Dilution Buffer to prepare final dilution 3000-fold and **mix well** (not to foam). Vortex is recommended.

Preparation of CSF samples:
Dilute CSF samples just prior to perform the test 100-fold with Dilution Buffer, e.g. 5 µl of sample + 495 µl Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Preparation of urine samples:
Dilute urine samples just prior to perform the test 10-fold with Dilution Buffer, e.g. 15 µl of sample + 135 µl of Dilution Buffer for singlets or 25 µl of sample + 225 µl of Dilution Buffer for duplicates. **Mix well** (not to foam). Vortex is recommended.

Stability and storage:
Do not store the diluted samples.
Serum and plasma samples should be stored at -20°C, or preferably at -70°C for long-term storage. See Chapter 13 for stability of serum and plasma samples if stored at 2-8°C, effect of sample matrix and effect off freezing/thawing on the Human Clusterin concentration.
CSF and urine samples should be stored at -70°C.

Ask for protocol at info@biovendor.com if assaying cell culture and cell lysate samples.

11. Assay Procedure

1) Pipet 100 µl of Standards, Quality Controls and diluted samples (for serum or plasma Dilution B), 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 300 rpm on an orbital microplate shaker.
3) Wash the wells 5-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 300 rpm on an orbital microplate shaker.
6) Wash the wells 5-times with Wash Solution (0.35 ml per well).
7) Add 100 µl of Streptavidin-HRP Conjugate solution.
8) Incubate the plate at room temperature (ca. 25°C) for 30 min, shaking at 300 rpm on an orbital microplate shaker.
9) Wash the wells 5-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 10 minutes following step 12.)

Note1: 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 Clusterin concentration of off-scale samples. The readings at 405 nm should not replace the on-scale readings 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 absorbent paper or 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 160**	Blank	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 80**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 40**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 20**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 10**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 5**	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 work sheet.

12. Calculations

Most microtiter plate readers perform automatic calculations of analyte concentration. The standard curve is constructed by plotting the absorbance (Y) of standards against the *log* of the known concentration (X) of standards, using the four-parameter function. Alternatively, the *logit log* function can be used to linearize the standard curve (i.e. *logit* of absorbance (Y) is plotted against *log* of the known concentration (X) of standards).

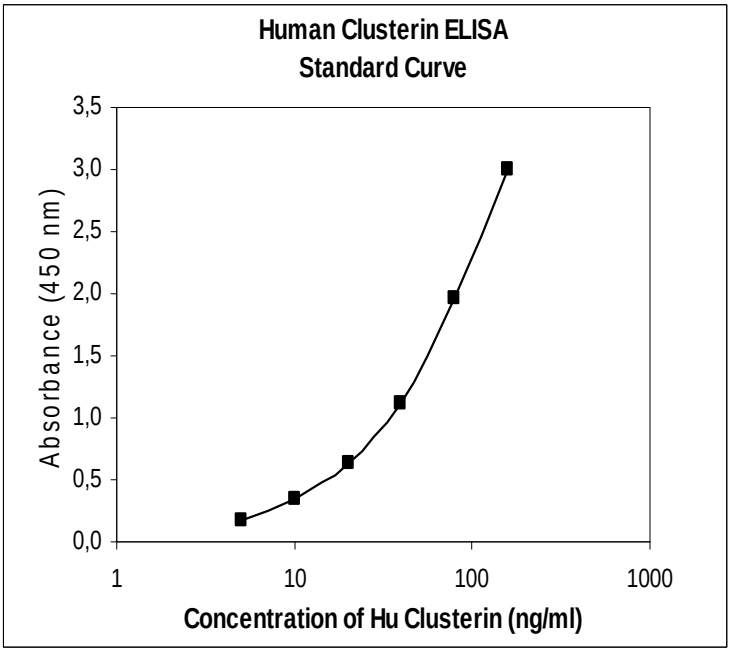

Figure 2: Typical Standard Curve for Human Clusterin

The measured Human Clusterin concentration in samples calculated from standard curve has to be multiplied by their respective dilution factor, because

samples have been diluted prior to the assay, e.g. 20 ng/ml (from standard curve) x 3 000 (dilution factor) = 60 000 ng/ml = 60 µg/ml.

13. Performance Characteristics

Typical analytical data obtained with the BioVendor Human Clusterin ELISA are presented in this chapter.

- **Sensitivity**

Limit of detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: A_{blank} + $3xSD_{blank}$) is calculated from the real human Clusterin values in wells and is: 0.5 ng/ml.

* Dilution Buffer is pipetted into Blank wells

- **Limit of assay**

Results exceeding human Clusterin level of 160 ng/ml should be repeated with more diluted samples. Dilution factor needs to be taken into consideration in calculating the Clusterin concentration.

Results from assaying of urine samples exceeding human Clusterin level of 40 ng/ml should be repeated with more diluted urine samples for correct values. Dilution factor needs to be taken into consideration in calculating the human Clusterin concentration.

- **Specificity**

The antibodies used in this ELISA are specific for human Clusterin.

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
Hamster	no
Horse	no
Monkey	yes
Mouse	no
Pig	no
Rabbit	no
Rat	no
Sheep	no

- **Precision**

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

Sample	*Mean (µg/ml)*	*Standard Deviation (µg/ml)*	*CV (%)*
1	68.0	4.8	7.0
2	103.6	5.6	5.4

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

Sample	*Mean (µg/ml)*	*Standard Deviation (µg/ml)*	*CV (%)*
1	62.4	5.3	8.5
2	95.8	6.7	7.0

- **Spiking Recovery**

Serum samples were spiked with different amounts of human Clusterin and assayed.

Sample	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	59.2	-	-
	139.6	139.2	100.3
	104.0	99.2	104.8
	81.6	79.2	103.0
2	75.6	-	-
	319.2	315.6	101.1
	196.8	195.6	100.6
	145.0	135.6	106.9

- **Dilution Linearity**

Serum samples were diluted with Dilution Buffer and assayed.

Sample	*Dilution*	***O**bserved (µg/ml)*	***E**xpected (µg/ml)*	*Recovery **O/E** (%)*
1	-	79.3	-	-
	2x	41.8	39.7	105.4
	4x	20.9	19.8	105.4
	8x	10.4	9.9	104.9
2	-	141.4	-	-
	2x	68.6	70.7	97.0
	4x	32.2	35.4	91.1
	8x	15.2	17.7	86.0

- **Effect of Sample Matrix (plasma/serum)**

Heparin, citrate and EDTA plasmas were compared to respective serum samples from the same 10 individuals.

However, we observed low correlation among serum and plasma human Clusterin values. Results are shown below.

Individuals	*Serum (µg/ml)*	*Plasma (µg/ml)*		
		Heparin	*Citrate*	*EDTA*
1	110.1	144.9	70.5	162.0
2	69.0	123.9	75.9	205.2
3	144.6	116.4	93.6	178.2
4	68.7	109.5	87.0	182.1
5	117.9	92.7	78.6	177.6
6	46.5	77.4	84.3	189.0
7	122.9	124.2	93.9	164.1
8	40.2	111.6	71.4	156.6
9	104.1	98.4	82.5	142.5
10	105.9	98.7	81.6	111.6
Mean	**93.0**	**109.8**	**81.9**	**166.9**

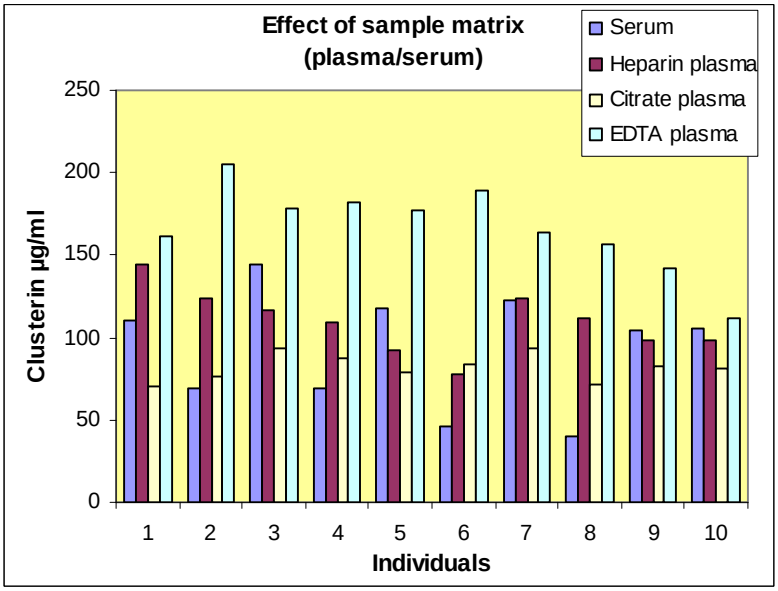

Figure 3: Human Clusterin levels measured using Human Clusterin ELISA from10 individuals using serum, heparin, citrate and EDTA plasma, respectively.

- **Effect of freezing/thawing**

No decline was observed in concentration of human Clusterin in serum samples after repeated (5x) freezing/thawing cycles.

Sample Number	*Number of f/t cycles*	*Serum (µg/ml)*	*Plasma (µg/ml)*		
			Heparin	*Citrate*	*EDTA*
1	1x	104.7	89.0	58.1	145.7
	3x	91.1	73.2	58.3	166.2
	5x	96.3	89.7	64.2	186.2
2	1x	78.2	56.6	51.2	145.7
	3x	95.2	87.4	57.3	149.5
	5x	84.1	72.9	56.1	186.6
3	1x	102.5	85.7	60.9	168.9
	3x	99.3	80.3	71.3	209.0
	5x	108.8	113.6	56.0	208.9

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

Samples should be stored at –20°C. Results of human Clusterin concentration in serum and plasma samples when stored at 2-8°C for 1 and 7 days are show below. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.3% and 0.1% concentration, respectively.

Sample Number	*Incubation Temp., Period*	*Serum (µg/ml)*	*Plasma (µg/ml)*		
			Heparin	*Citrate*	*EDTA*
1	-20°C	99.6	89.6	72.9	199.4
	2-8°C, 1 days	68.2	104.0	63.8	189.9
	2-8°C, 7 days	72.6	158.1	65.1	209.7
2	-20°C	71.6	58.9	48.6	188.0
	2-8°C, 1 days	101.7	81.3	63.0	178.8
	2-8°C, 7 days	102.3	99.0	84.6	235.5
3	-20°C	71.9	72.5	54.0	155.6
	2-8°C, 1 days	62.4	87.1	54.6	165.5
	2-8°C, 7 days	75.1	97.4	58.7	137.0

14. Definition of the Standard

The recombinant human Clusterin is used as the Standard. The recombinant Human Clusterin, produced in HEK 293 cells, is dimeric 75-80 kDa protein.
1 ng/ml of recombinant Human Clusterin corresponds to 2 U/ml of human serum based Standard, which was used in previous Biovendor Human Clusterin ELISA.

15. Preliminary population and clinical data

The following results were obtained when serum samples from 200 unselected donors (100 female + 100 male) 4-80 years old were assayed with the Biovendor Human Clusterin ELISA in our laboratory.

- **Normal value**

Population Mean (mean +/- SEM): 95.0 +/- 1.5 µg/ml
Normal Range (mean +/- 2SD): 95.0 +/- 42.2 µg/ml

However, it is recommended to establish a normal value of control serum for each laboratory. The presented reference should be regarded as guideline.

- **Age dependent distribution of human Clusterin**

Sex	Age years	n	Mean	SD	Min	Max
			Clusterin µg/ml			
Male	4-19	17	89.9	16.4	62.5	106.5
	20-29	20	88.6	19.8	52.5	132.5
	30-39	15	96.6	21.6	56.0	147.0
	40-49	15	91.9	20.9	67.5	131.5
	50-59	15	88.1	15.4	64.0	107.0
	60-80	18	97.8	20.7	71.5	135.0
Female	3-19	17	93.0	23.0	64.0	157.5
	20-29	20	97.2	14.8	51.5	113.0
	30-39	15	99.6	17.5	64.5	133.5
	40-49	15	108.1	22.4	80.5	164.0
	50-59	16	95.2	25.9	59.0	127.5
	60-80	17	94.9	35.0	54.0	164.5

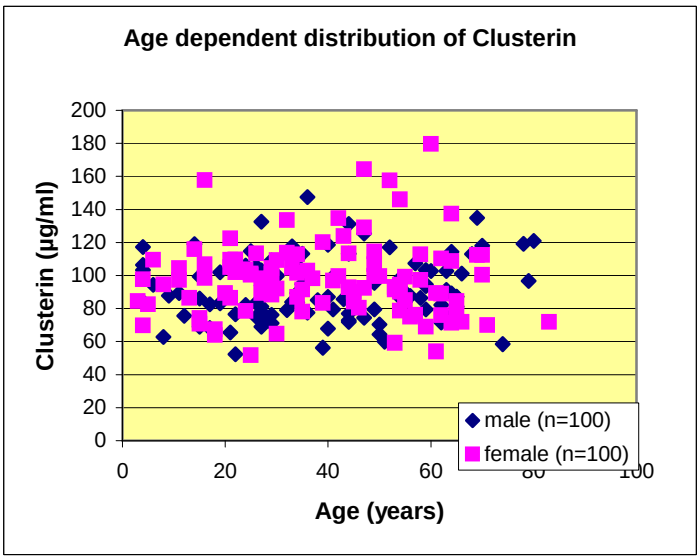

Figure 4: Human Clusterin concentration plotted against donor age.

16. Method Comparison

The BioVendor Human Clusterin ELISA was not compared to the other commercial immunoassays.

17. 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
- Improper wavelength when reading absorbance

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
- Incubation temperature over 30°C

3/ High coefficient of variation (CV)

Possible explanation:

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

18. References

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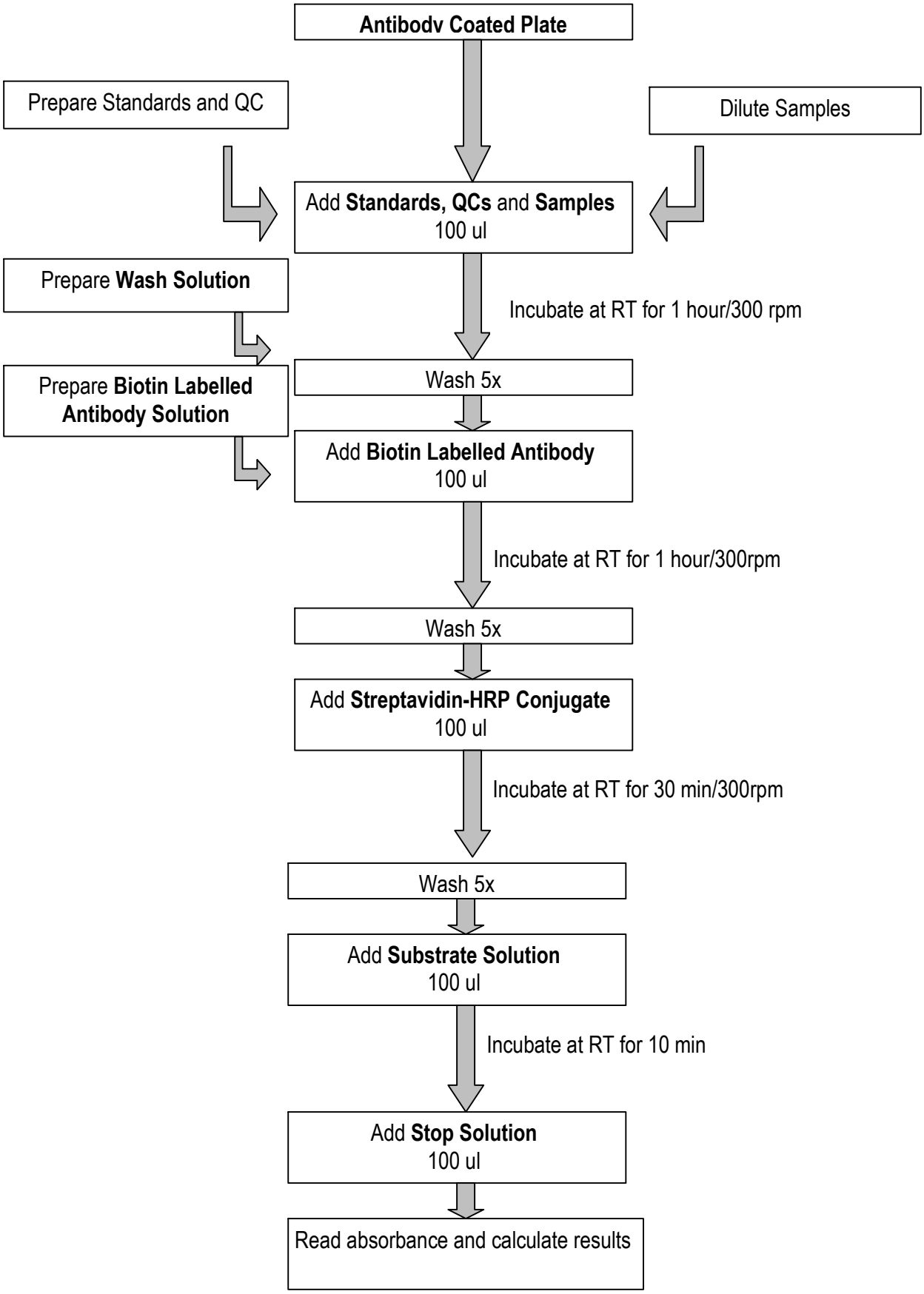
For more references on this product
see our WebPages at www.biovendor.com

19. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Biological hazard
	Expiry date
2°C 8°C	Storage conditions
5 PP	Identification of packaging materials

Assay Procedure Summary



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A												
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Notes: