

ENG

Instructions for Use:
RAT CLUSTERIN ELISA

Catalogue number:
RBL025R

For research use only!

 **BioVendor**
R&D[®]



BioVendor – Laboratorní medicína a.s.

Karásek 1767/1, 621 00 Brno, Czech Republic

+420 549 124 185

info@biovendor.com

sales@biovendor.com

www.biovendor.com

1. INTENDED USE	3
2. STORAGE, EXPIRATION	3
3. INTRODUCTION	3
4. TEST PRINCIPLE	3
5. PRECAUTIONS	4
6. REAGENT SUPPLIED	4
7. MATERIAL REQUIRED BUT NOT SUPPLIED	4
8. PREPARATION OF REAGENTS	4
9. PREPARATION OF SAMPLES	5
10. ASSAY PROCEDURE	6
11. PERFORMANCE CHARACTERISTICS	6
12. DEFINITION OF THE STANDARD	7
13. REFERENCE RANGE	7
14. CALCULATION	8
15. REFERENCES	9
16. EXPLANATION OF SYMBOLS	10
17. ASSAY PROCEDURE - SUMMARY	11

HISTORY OF CHANGES

Previous version	Current version
ENG.001.A	ENG.002.A
Chapter 6: Master Standard – 2 vials	

1. INTENDED USE

Enzyme Immunoassay for the quantitative determination Clusterin in rat serum and rat urine.

2. STORAGE, EXPIRATION

- The kit must be stored at 2 – 8 °C.
- The opened components can be stored for one week at 2 – 8 °C.

3. INTRODUCTION

Clusterin (Apolipoprotein 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.

Clusterin is highly conserved across species, showing 70-80% identity at the amino acid level amongst mammals, and numerous variants and isoforms have been described. 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 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 proposed, 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.

Interesting study determine that urinary clusterin levels in the rat correlate with severity of tubular damage and may help to differentiate between glomerular and tubular injuries.

4. TEST PRINCIPLE

In the Rat Clusterin ELISA kit, standards and samples are incubated in microtitration wells pre-coated with polyclonal anti-rat clusterin antibody. After 60 minutes incubation followed by washing, biotin-labelled polyclonal anti-rat 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 and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of rat Clusterin. A standard curve is constructed by plotting absorbance values against clusterin concentrations of standards and concentrations of unknown samples are determined using this standard curve.

5. PRECAUTIONS

- For research use only
- For professional laboratory use
- The reagents with different lot numbers should not be mixed
- To prevent cross sample contamination, use disposable labware and pipette tips
- To protect laboratory stuff, wear protective gloves and protective clothing
- The substrate solution should remain colourless, keep it protected from light
- The test should be performed at standard laboratory conditions (temperature 25 °C ±2 °C).

6. REAGENT SUPPLIED

Item	Qty.
Antibody Coated Microtiter Plate	96 wells
Streptavidin-HRP Conjugate	13 ml
Biotin Labelled Antibody Conc. (50x)	265 µl
Biotin-Ab Diluent	13 ml
Master Standard (lyophilized)	2 vials
Quality Control A (lyophilized)	1 vial
Quality Control B (lyophilized)	1 vial
Dilution Buffer	2x13 ml
Wash Buffer 15x conc.	50 ml
Substrate Solution	13 ml
STOP Solution	13 ml

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Glassware and test tubes
- Microtiter plate washer
- Precision pipettes (various volumes) with tips
- Orbital shaker
- Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

8. PREPARATION OF REAGENTS

Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination. All reagents and samples should be allowed to reach the temperature 25 °C ±2 °C.

8.1 Preparation of Standards

Rat Clusterin 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 rat Clusterin in the stock solution is 128 ng/ml.

Prepare set of Standard solution as follows:

Use the Master Standard to produce a dilution series (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 128 ng/ml (lyophilised)	See CoA	128 ng/ml
Std2	300 µl of Std1	300 µl	64 ng/ml
Std3	300 µl of Std2	300 µl	32 ng/ml
Std4	300 µl of Std3	300 µl	16 ng/ml
Std5	300 µl of Std4	300 µl	8 ng/ml
Std6	300 µl of Std5	300 µl	4 ng/ml
Std7	300 µl of Std6	300 µl	2 ng/ml
Blank	-	200 µl	0 ng/ml

8.2 Preparation of Quality Control A and B

Reconstitute the lyophilized Quality Controls in dilution buffer, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min. The reconstituted Quality controls are ready to use, do not dilute them.

8.3 Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 ml of Wash Buffer 15x conc. to 700 ml of deionized/ distilled water (dH₂O). Mix well. Store at 4 °C for two weeks or at -20 °C for long term storage.

8.4 Biotin Labelled Antibody 1x

Prepare a working solution of Biotin-labelled antibody by adding 265 µl of Biotin-labelled antibody 50x conc. to 13 ml of Biotin-Ab Diluent. Mix well.

9. PREPARATION OF SAMPLES

Rat serum or urine may be used with this assay. It is recommended to assay not-frozen samples. For long-term storage the serum and plasma samples should be frozen at minimum -70 °C and the urine samples should be frozen in protective medium at minimum -70 °C.

Recommended dilution of samples is 1:2000. It is recommended to use the two-step dilution. Dilution A (40x) for both singlets and duplicates: 5 µl of samples + 159 µl of Dilution Buffer. Dilution B (50x): 5 µl of Dilution A + 245 µl of Dilution Buffer, for both singlets and duplicates; Mix well, vortex is recommended.

Recommended dilution of urine is 1:10, i.e., 20 of samples + 180 µl of Dilution Buffer, for singlets, 30 of samples + 270 µl of Dilution Buffer, for duplicates.

Do not store the diluted samples. Do not store the diluted samples.

10. ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 μ l of set of Standards, Quality Controls, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25 °C \pm 2 °C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 μ l/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 μ l of Biotin Labelled Antibody into each well. Incubate for **1 hour** at 25 °C \pm 2 °C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 μ l of HRP-labelled Antibody Conjugate into each well. Incubate for **30 minutes** at 25 °C \pm 2 °C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 μ l Substrate solution, incubate for **10 min**, at 25 °C \pm 2 °C. Avoid exposure to the light during this step.
9. Pipette 100 μ l of STOP solution.

Read the signal at 450 or 450/630 nm within 15 min.

11. PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:2000 as recommended and assayed. The results are multiplied by the dilution factor.

11.1 Sensitivity

The limit of detection, defined as a concentration of rat clusterin giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.7 ng/ml of sample.

11.2 Precision

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

Sample	Mean (μ g/ml)	SD (μ g/ml)	CV (%)
1	11.6	0.5	4
2	8.6	0.5	6

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

Sample	Mean (μ g/ml)	SD (μ g/ml)	CV (%)
1	29.3	1.6	5.5
2	37.6	2.3	6.2

11.3 Accuracy

11.3.1 Dilution linearity

Sample	Dilution	Measured concentration (µg/ml)	Expected concentration (µg/ml)	Yield (%)
Serum 1		7.0	-	-
	2x	3.6	3.5	98
	4x	1.8	1.7	96
	8x	0.8	0.9	106
Serum 2		8.0	-	-
	2x	4.4	4.0	92
	4x	2.2	2.0	90
	8x	1.0	1.0	105

11.3.2 Spiking Recovery

Sample	Spike (ng/ml)	Measured concentration (µg/ml)	Expected concentration (µg/ml)	Yield (%)
Serum 1	-	26.9	-	-
	0.5	130.9	129.3	101
	1.0	76.9	78.1	99
	2.0	54.0	52.5	103
Urine 1	-	37.2	-	-
	0.5	90.6	101.2	90
	1.0	65.2	69.2	95
	2.0	52.0	53.2	98

12. DEFINITION OF THE STANDARD

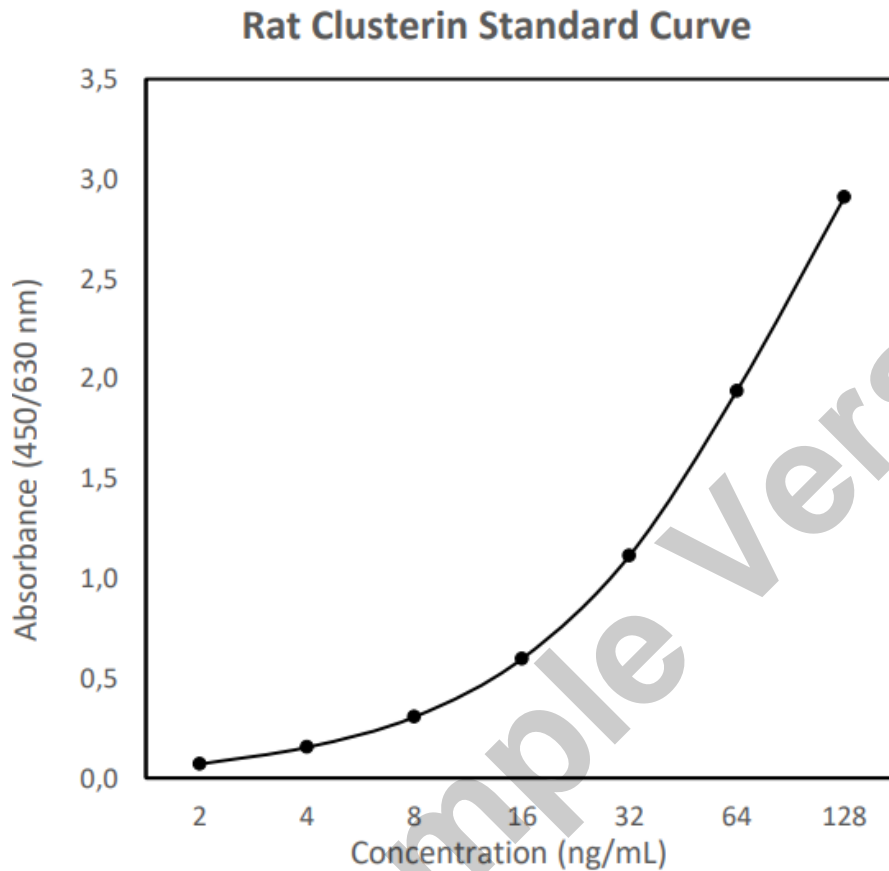
The recombinant rat clusterin is used as the Standard. The recombinant rat clusterin, produced in *E. coli*, is 26.5 kDa protein containing 215 amino acid residues of the rat clusterin and 25 additional amino residues. The amino acid sequence of the recombinant rat cluster is 100% homologous to the amino acid sequence 146-360 of the rat clusterin precursor.

13. REFERENCE RANGE

It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for rat clusterin levels with the assay.

14. CALCULATION





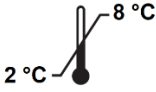




The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



15. REFERENCES

1. Hidaka S, Kränzlin B, Gretz N, Witzgall R: Urinary clusterin levels in the rat correlate with the severity of tubular damage and may help to differentiate between glomerular and tubular injuries. *Cell Tissue Res*, 2002 Oct, 310:289-296
2. Jones SE, Jomary C: Molecules in focus Clusterin. *The International J of Bioch & Cell Biol*, 2002 May, 34: 427-431
3. DeMattos RB, O'dell MA, Parsadanian M, Taylor JW, Harmony JAK, Bales KR, Paul SM, Aronow BJ and Holtzman DM: Clusterin promotes amyloid plaque formation and is critical for neurotoxicity in a mouse model of Alzheimer's disease. *Proc Natl Acad Sci*, 2002 Aug, 99: 10843-10848
4. Chen X, Halberg RB, Ehrhardt WM, Torrealba J and Dove WF: Clusterin as a biomarker in murine and human intestinal neoplasia. *Proc Natl Acad Sci*, 2003 Aug, 100: 9530-9535
5. Min BH, Kim BM, Lee SH, Kang SW, Bendayan M. and Park IS: Clusterin expression in the early process of pancreas regeneration in the pancreatectomized Rat. *The J of Histochem & Cytochem*, 2003, 51(10): 1355-1365
6. Trougokos IP, Gonos ES: Functional analysis of clusterin/apolipoprotein J in cellular death induced by severe genotoxic stress. *Ann NZ Acad Sci*, 2004 Jun, 19:206-210
7. Krijnen PAJ, Cillessen SAGM, Manoe R, Muller A, Visser CA, Meijer CJLM, Musters RJP, Hack CE, Aarden LA, and Niessen HWM: Clusterin: a protective mediator for ischemic cardiomyocytes? *Am J Physiol Heart Circ Physiol* 2005; 289:H2193-H2202
8. Kim BM, Kim SY, Lee S, Shin YJ, Min BH, Bendayan M, Park IS: Clusterin induces differentiation of pancreatic duct cells into insulin-secreting cells. *Diabetologia* 2006; 49:311-320
9. Kruger S, Mahnken A, Kausch I, Feller AC: Value of Clusterin immunoreactivity as a predictive factor in muscle-invasive urothelial bladder carcinoma. *Urology* 2006; 67:105-109
10. Rodriguez-Pineiro AM, De la Cadena MP, Lopez-Saco A, and Rodriguez-Berrocal FJ: Differential Expression of serum clusterin isoforms in colorectal cancer. *Mol. And Cel. Proteomics* 2006; 5:1647-1657
11. Strochi P, Smith MA, Perry G, Tamagno E, Danni O, Pession A, Gaiba A, Dozza B: Clusterin up-regulation following sub-lethal oxidative stress and lipid peroxidation in human neuroblastoma cells. *Neurobiol. of Aging* 2006; 27:1588-1594
12. Ishii A, Sakai Y, and Nakamura A: Molecular pathological evaluation of clusterin in a rat model of unilateral ureteral obstruction as a possible biomarker of nephrotoxicity. *Toxicologic Pathology* 2007; 35:376-382
13. Stoop MP, Dekker LJ, Titulaer MK, Burgers PC, Sillevius Smitt PAE, Luijckx TM, and Hintzen RQ: Multiple sclerosis-related identified in cerebrospinal fluid by advanced mass spectrometry. *Proteomics* 2008; 8:0000-0000

16. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
 <p data-bbox="256 1182 466 1205">www.biovendor.com</p>	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks

17. ASSAY PROCEDURE - SUMMARY

Add 100 μL of Standards, diluted QCs and Samples to the wells



Incubate 1 hour at 25°C, shaking 300 rpm

3-times wash the wells (350 μL /well)



Add 100 μL of Biotin-labelled Antibody to the wells



Incubate for 1 hour at 25°C, shaking at 300 rpm

3-times wash the wells (350 μL /well)



Add 100 μL of SAV-HRP to the wells



Incubate for 30 min at 25°C, shaking at 300 rpm

3-times wash the wells (350 μL /well)



Add 100 μL of Substrate Solution to the wells



Incubate for 15 min in the dark at 25°C, NO shaking

Add 100 μL of Stop Solution to the wells



Read the signal at 450 nm (450/630 nm) within 15 min



BioVendor – Laboratorní medicína a.s.

Karásek 1767/1, 621 00 Brno, Czech Republic

+420 549 124 185

info@biovendor.com

sales@biovendor.com

www.biovendor.com



FOLLOW US