Rat Clusterin ELISA

Cat. No.: BA2011

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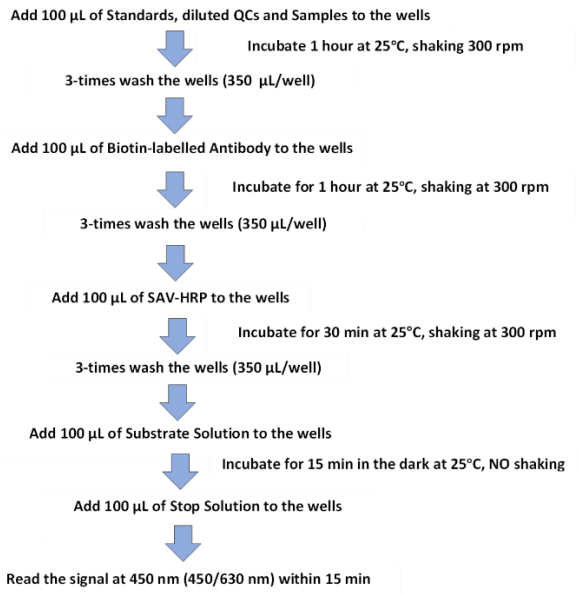
Enzyme Immunoassay for the quantitative determination Clusterin in rat serum and rat urine.

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.
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.

PRINCIPLE OF RAT CLUSTERIN ELISA

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 Ccusterin. 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.



Kit Contents

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

MATERIAL REQUIRED BUT NOT SUPPLIED

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

WARNINGS AND PRECAUTIONS

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

STORAGE CONDITIONS

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

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.

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 CofA	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

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.

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 (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

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.

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.

ASSAY PROCEDURE

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

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

PERFORMANCE CHARACTERISTICS

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

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.

2. Precision

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

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	11.6	0.5	4
2	8.6	0.5	6

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

Sample	Mean (µg/mL)	SD (µg/mL)	CV (%)
1	29.3	1.6	5.5
2	37.6	2.3	6.2

3. Accuracy

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

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

Definition of the standard

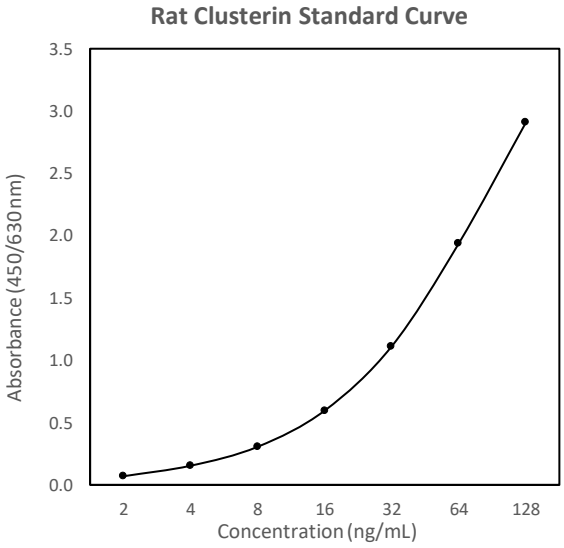
The recombinant rat clusterin is used as the Standard. The recombinant rat clusterin, produced in E. coli, is 26.5 kDa protein containing 215 amino acid residues of the rat clusterin and 25 additional amino residues. The amino acid sequence of the recombinant rat cluster is 100% homologous to the amino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

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.

Typical standard curve

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.



RESOURCES

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