KAMIYA BIOMEDICAL COMPANY

Rat Clusterin ELISA

For the quantitative determination of clusterin in rat serum and plasma.

Cat. No. KT-432

For Research Use Only.

PRODUCT INFORMATION

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PRODUCT

The **K-ASSAY**® Rat Clusterin ELISA is an enzyme immunoassay for the quantitative determination of clusterin in rat serum and plasma. For research use only.

INTRODUCTION

Clusterin, also referred to as apolipoprotein J, sulfated glycoprotein-2, glycoprotein III and testosterone-repressed prostate message-2, is a glycoprotein of 70 - 80 kDa, composed of one α-subunit and one β-subunit, derived from proteolytic cleavage of a precursor peptide. Clusterin is expressed in many tissues and is found in serum, seminal fluid and urine. It has been identified as a potential biomarker of various forms of renal injury and prostate disease.

PRINCIPLE

The **K-ASSAY**® Rat Clusterin ELISA uses two different affinity purified antibodies. One is used for solid phase immobilization (on the microtiter wells). The second is conjugated to horse radish peroxidase (HRP). The samples (diluted serum, plasma or urine) are mixed in the microtiter wells with HRP conjugated antibody and clusterin is sandwiched between the solid phase and HRP-conjugated antibodies. After incubation on a plate shaker for one hour at room temperature the wells are washed to remove unbound HRP-conjugated antibodies. A solution of tetramethylbenzidine (TMB), an HRP substrate, is then added and incubated for 20 minutes, resulting in the development of blue color. The color development is stopped by the addition of 1N HCl changing the color to yellow. The concentration of clusterin is proportional to the absorbance at 450 nm.

COMPONENTS

- Anti-rat clusterin coated wells (1 plate, 96 wells)
- Rat Clusterin Calibrator: Lyophilized clusterin (reconstitute with 0.20 mL H_2O)
- Diluent (10X), 25 mL
- Wash Solution (20X), 50 mL
- Anti-clusterin HRP Conjugate, 11 mL
- TMB Reagent (HRP substrate solution), 11 mL
- Stop Solution (1N HCl), 11 mL

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or de-ionized water
- Pipettes: P-10, P-200 & P-1000 or equivalent
- Disposable pipette tips
- Plate reader capable of reading OD at 450 nm
- Vortex mixer
- Absorbent paper
- Graph paper or appropriate PC graphing software
- Polypropylene microcentrifuge tubes (1.5 mL)

DILUENT PREPARATION

The diluent is provided as a 10X stock. Prior to use estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10X stock with nine (9) volumes of distilled or de-ionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20X stock. Prior to use dilute the contents of the bottle (50 mL) with 950 mL of distilled or de-ionized water.

CALIBRATOR PREPARATION

1. Reconstitute the lyophilized clusterin stock by addition of 200 µL of de-ionized or distilled water. Mix gently several times over a period of 5 minutes. The concentration of clusterin in the reconstituted stock is indicated on the vial label.
2. Label 8 polypropylene tubes as 250, 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 ng/mL.
3. Into the tube labeled 250 ng/mL, pipette the volume of 1x diluent detailed on the clusterin stock vial label. Then add the indicated volume of clusterin stock (shown on vial label) and mix gently. This provides the 250 ng/mL calibrator.
4. Pipette 0.25 mL of clusterin diluent into the tubes labeled 125, 62.5, 31.25, 15.6, 7.8, 3.9 and 0 ng/mL.
5. Prepare a 125 ng/mL calibrator by diluting and mixing 0.25 mL of 250 ng/mL calibrator with 0.25 mL of diluent in the tube labeled 125 ng/mL. Similarly prepare the 62.5, 31.25, 15.6, 7.8 and 3.9 ng/mL calibrators by serial dilution.

NOTE: The reconstituted clusterin calibrator should be frozen immediately after use. It remains stable in frozen form for at least 1 month at -20°C and 6 months at -70°C. Discard the working 250-3.9 ng/mL calibrators after use.

SAMPLE COLLECTION AND PREPARATION

Serum or plasma should be prepared as quickly as possible after blood collection. If samples cannot be assayed immediately they should be frozen at -70°C and thawed only once prior to use.
Samples must not contain azide because this inactivates the HRP conjugate.
Clusterin is generally present in normal rat serum or plasma at a concentration of ~ 10 µg/mL. In order to obtain values within the range of the calibration curve we suggest that samples be diluted 100 fold by mixing 3 µL of sample with 297 µL of 1X diluent.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. **Dispense 100 µL of samples into the appropriate wells (diluted samples must be added before the HRP conjugate).**
3. Dispense 100 µL of HRP conjugate into each well.
4. Incubate on an orbital shaker (150 rpm) at room temperature (18-25°C) for 60 minutes.
5. Remove the incubation mixture using a plate washer or by flicking plate contents into a Bio-waste container.
6. Wash and empty the microtiter wells 6 times with wash solution. This may be performed using either a plate washer (400 µL/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
7. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
8. Dispense 100 µL of TMB Reagent into each well.
9. Incubate on an orbital shaker (150 rpm) at room temperature for 20 minutes.
10. Stop the reaction by adding 100 µL of Stop Solution to each well.
11. Gently mix until all the blue color changes to yellow.
12. Read absorbance at 450 nm with a plate reader within 15 minutes. **Please Note: Due to plate reader differences, the high calibrator absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead.**
13. If absorbance values exceed the high calibrator, the samples should be appropriately diluted and re-determined.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the calibrators and samples.
2. Construct a calibration curve by plotting A_{450} values obtained for each reference calibrator against its concentration in ng/mL on graph paper, with absorbance on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the A_{450} values for each sample, determine the corresponding concentration of clusterin (ng/mL) from the calibration curve.
4. Multiply the derived clusterin value by the dilution factor to obtain the concentration in the original sample.
5. If available, graphing software may be used to analyze the data. Depending on the range of the calibration curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, calibration curves may be generated using a point-to-point fit.


TYPICAL CALIBRATION CURVE

Result of a typical calibration curve with A_{450} plotted on the Y axis against clusterin concentration on the X axis is shown below.
NOTE: This calibration curve is for the purpose of illustration only.

Clusterin (ng/mL)	Absorbance (450 nm)
250	2.407
125	1.810
62.5	1.190
31.25	0.768
15.6	0.481
7.8	0.287
3.9	0.210
0	0.133

Typical Rat Clusterin Calibration Curve
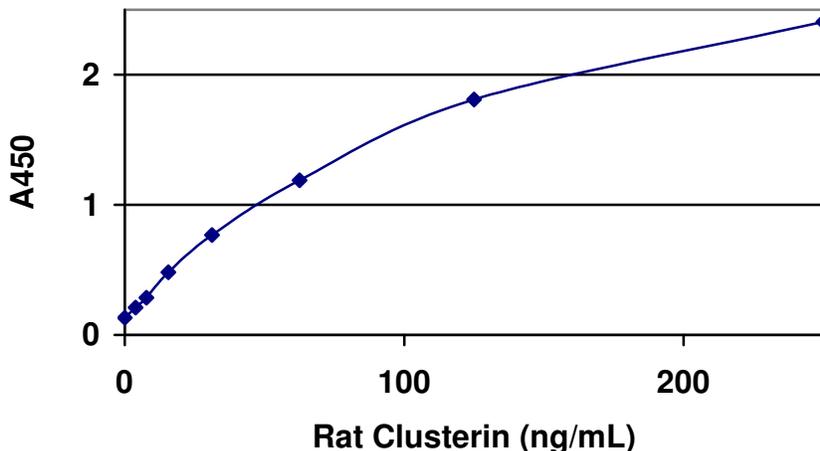
STORAGE
Store the kit at 4°C. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.
The kit expiration date is indicated on the box label.

WARNINGS AND PRECAUTIONS
- Avoid contact with 1N HCl (stop solution). It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
- Do not use reagents after expiration date and do not mix or use components from different kits.
- Replace caps on reagents immediately. Do not switch caps.
- Do not pipette reagents by mouth.

LIMITATIONS OF THE PROCEDURE
1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
2. Urine sample can be assayed with this kit but we have not been able to fully validate the ELISA for use with urine due to a lack of positive control samples. Studies with clusterin-spiked urine samples indicate that best results are obtained if urine samples are either (i) diluted at least 50-fold in 1X diluent, or (ii) dialyzed against a 200-fold volume excess of de-ionized water and mixed with an equal volume of 2X diluent prior to assay. Either of these methods eliminates interfering factors. Use of this kit for measurement of urinary clusterin levels should be performed entirely at the discretion of the researcher.

FOR RESEARCH USE ONLY

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