

RAT CLUSTERIN ELISA KIT

Life Diagnostics, Inc., Cat. No. 3300-2

ELISA FOR DETERMINATION OF RAT CLUSTERIN

STORAGE CONDITIONS

Store the kit at 2-8°C. Keep the microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

EXPIRATION

The kit expiration date (six months from the date of shipment) is indicated on the package label

BACKGROUND

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 OF THE ASSAY

The 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 a blue color. The color development is stopped by addition of 1N HCl changing the color to yellow. The concentration of clusterin is proportional to the absorbance at 450 nm.

REAGENTS AND MATERIALS PROVIDED

- Anti clusterin-coated wells (1 plate, 96 wells)
- Clusterin Stock: Lyophilized clusterin (reconstitute with 0.20 ml H₂O)
- 10x Diluent (25 ml)
- 20x Wash solution (50 ml)
- Anti-clusterin HRP Conjugate (11 ml)
- TMB Reagent (11 ml): HRP substrate solution
- Stop Solution (11 ml): 1N HCl

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized 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)

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.

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 deionized 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 deionized water.

STANDARD PREPARATION

1. Reconstitute the lyophilized clusterin stock by addition of 200 μ l 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 the clusterin stock vial label) and mix gently. This provides the 250 ng/ml standard.
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 standard by diluting and mixing 0.25 ml of the 250 ng/ml standard with 0.25 ml of diluent in the tube labeled 125 ng/ml. Similarly prepare the 62.5, 31.25, 15.6, 7.8, 3.9 ng/ml standards by serial dilution.

NOTE: The reconstituted clusterin stock 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 standards 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 μ g/ml. In order to obtain values within the

range of the standard curve we suggest that samples be diluted 100 fold by mixing 3 μ l of sample with 297 μ l of 1x diluent.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. **Dispense 100 μ l of samples into the appropriate wells (diluted samples must be added before the HRP conjugate).**
3. Dispense 100 μ l 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 the 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 μ l/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 μ l 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 μ l 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 standard 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 standard, the samples should be appropriately diluted and re-determined.

CALCULATION OF RESULTS

1. Calculate the mean absorbance value (A_{450}) for the standards and samples.
2. Construct a standard curve by plotting the A_{450} values obtained for each reference standard 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 standard 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 standard 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, standard curves may be generated using a point-to-point fit.

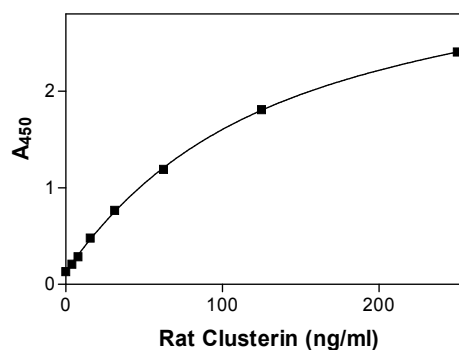
EXAMPLE OF STANDARD CURVE

Results of a typical standard curve with A_{450} plotted on the Y axis against clusterin concentrations on the X axis are shown below.

NOTE: This standard 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

Representative Rat Clusterin Standard Curve



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 samples 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.