**Clusterin-CIC Proceptor™ ELISA Kit Data Sheet**  
*Product Code CR-001*

**INTRODUCTION**  
Clusterin (apolipoprotein J) is an extracellular, highly conserved and heavily glycosylated, disulfide-linked heterodimer with an apparent molecular mass of 75–80 kDa. Clusterin is constitutively expressed in almost all mammalian tissues and in different cell types. Furthermore, clusterin is found in all body fluids, including plasma, seminal plasma, urine, and cerebrospinal fluid. It has a chaperone-like activity. Low levels of clusterin are expressed in tumor cells undergoing cell death by apoptosis. Clusterin has anti-proliferative activity and has been shown to inhibit proliferation of the prostate cancer cells.

Clusterin has also been reported to play a role in a wide variety of processes and has been shown to interact with several components of the soluble C5b-9 (sC5b-9) also referred to as soluble membrane attack complex (sMAC) of complement. Expression of clusterin is upregulated in atherosclerosis, myocarditis, oxidative stress and heat shock, Alzheimer’s disease, several cancers, and after injury in general. Clusterin deposition has been reported in acute myocardial infarction (AMI), where it is co-localized with complement factors C1q, C4, C3d, and C9 on cardiomyocytes within the infarcted area.

Proceptor™ (CR-001) ELISA kit is the only available assay, which measures Clusterin bound to the circulating immune complexes (CICs). The kit provides a better indicator of immune status than the measurement of total serum clusterin, since the assay measures clusterin, which is actively involved in immune physiological process.

**ASSAY PRINCIPLE**  
The assay is based on unique capture of ICs by ProGen’s proprietary receptors. These purified receptors are pre-coated onto the microtiter plate. The samples at appropriate concentration are pipetted into the wells. CICs present in the serum then bind to the receptors. After washing away any unbound material, a polyclonal antibody to clusterin is added to all wells of the plate and allowed to interact with the clusterin bound to the CICs. Again the plates are washed to remove the unbound anti-clusterin antibody. The wells are then filled with secondary antibody-enzyme reagent specific for the anti-clusterin antibody. After incubation TMB substrate solution is added to the wells. Intensity of color developed is proportionate to the amount of Clusterin bound to the CICs.

**REAGENTS**  
**Receptor Microplate (7000104):** Polystyrene microtiter plate (12X8 wells) coated with receptors. Rows A1-H1 and A2-H2 are coated with varying concentration of purified Clusterin. The standards are coated from 1.56 to 200 ng/ml in doubling dilution.

**Anti-Clusterin Antibody (7000104) (Rabbit):** One vial containing 1.2 ml (10X) of anti-Clusterin antibody (purified globulin fraction) with stabilization solution and preservative.

**HRP Conjugate (1000700) (Goat):** One bottle containing 12 ml of secondary antibody against clusterin antibody. Purified immunoglobulin fraction conjugated to Horseradish peroxidase (HRP).

**Color Development Reagent (1000901):** One bottle containing 12 ml of stabilized chromogen TMB (tetramethylbenzidine).

**Stop Solution (1000801):** One bottle containing 7 ml of 0.25 N Sulfuric Acid.

**Wash Buffer (1000601):** Two bottles each containing 50 ml of 10X PBS/Tween-20. Prepare a working solution of wash buffer by adding 50 ml of 10X wash buffer to 450 ml of deionized water to make 500 ml of 1X wash buffer. The 1X wash buffer can be used to wash the plate or to dilute samples.

**SAMPLE COLLECTION**  
Serum – Collect blood in serum separator tube and allow clotting for 30 minutes to 60 minutes before centrifugation at 1500 g. Separate, the serum and assay immediately or aliquot in small volumes for future use.

Plasma – Collect blood using heparin as an anticoagulant. Centrifuge at 1500 g within 30 minutes for future use.

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SAMPLE STORAGE
Use serum or heparinized plasma samples immediately after collection or store sample preferably below -70°C for future use. Avoid repeated freeze-thaw cycles. Sample stored at -20°C can also be used.

Note: Hemolyzed samples and samples exposed to higher temperatures are not suitable for measurement of Clusterin.

SAMPLE PREPARATION
Serum or plasma is diluted 1:20 to 1:40 using 1X wash buffer. For samples having higher concentration of Clusterin bound should be diluted appropriately and assayed again so the measured optical density falls within the standard curve.

Note: The reagents should be brought to room temperature before using the test kit. Bring all solutions to room temperature (20-25°C).

ASSAY PROCEDURE
Any debris observed should be removed by centrifugation at 1500g for 10 minute at room temperature from the samples prior to sample dilution.

1. Dilute the samples to appropriate dilution. We recommend 1:20 to 1:40 dilution of the samples.
2. Leave the wells marked standards Lane A1-A2 to H1-H2 as such in first step. These wells do not receive any solution in the first step and are covered with film.
3. Wash the wells in the rest of the plate with 200 µl of 1X wash buffer for two minutes.
4. Pipette 100 µl of sample per well. It is recommended to run the assay in duplicate wells.
5. Incubate the plate with samples for ninety minutes at room temperature (20 to 25°C).
6. Wash 4-times with 300 µl of wash buffer using a squirt bottle or automated plate washer. Allow buffer to stay in the wells for 2 minutes between each wash.
7. Add 100 µl of anti-Clusterin antibody (diluted to 1X with wash buffer) to each well and incubate the plate for sixty minutes at room temperature (20 to 25°C).
8. Repeat wash as in step 6.
9. Add 100 µl of HRP-Conjugate and incubate for sixty minutes at room temperature (20 to 25°C).
11. Add 100 µl of color development reagent and stop the reaction by addition of 50 µl of stop reagent when desired optical density is reached. During the development gently tap the plate occasionally.
12. Read the plate using a microplate reader at 450 nm.
13. Plot the standards values against the known concentration and using the linear equation calculate the sample values (y = a + bX).
14. Use the linear portion of the curve for calculating the values.

It is preferred to use plate shaker during incubations to obtain uniform binding.

CALCULATION OF RESULTS
Make a spreadsheet and enter the data. Average the duplicate reading for each standard, control and sample. Subtract the average zero standard optical density; if the function is available in the machine the plate reader can do this.

Create a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw the best-fit curve through the points on the curve. Discard the values, which distort the curve or add undesired error. Calculate the slope and intercept use these values in a linear equation y = a+bX to calculate the sample values.

LIMITATION OF ASSAY
THE ASSAY IS FOR RESEARCH USE ONLY AND NOT FOR USE IN DIAGNOSTIC PROCEDURES.

• The samples should be clear and prepared as described.
• It is important that the sample values fall within the linear part of the standard curve.
• The kit should be used within the expiration date.
• Variations in operator, pipetting technique, washing technique, incubation time, incubation temperature, kit age and presence of interfering agents in the serum and plasma samples may cause variation in binding.
## Standard Curve for Clusterin

<table>
<thead>
<tr>
<th>Conc. ng/ml</th>
<th>OD 450 nm</th>
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<tbody>
<tr>
<td>1.5625</td>
<td>0.0645</td>
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<tr>
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<td>25</td>
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<tr>
<td>100</td>
<td>1.586</td>
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<tr>
<td>200</td>
<td>2.717</td>
</tr>
</tbody>
</table>

**Slope**: 0.01373864  
**Intercept**: 0.048189

Use the linear portion of the curve for calculation.

The insert represents a typical standard curve; however some variation may occur due to the laboratory and user techniques.

**Store Refrigerated**

**Batch No.**:

**Expiry Date**:

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