**Vitronectin-C5b-9 (sMAC)**

Proceptor™ ELISA Kit Data Sheet (Product Code CR-004)

**INTRODUCTION**

Vitronectin is a 70-kDa glycoprotein present in plasma at a concentration of 50-100µg/ml. Vitronectin is a major cell attachment promoting protein that is cleaved by Granzyme B at RGD sequence. Elevated levels of Vitronectin bound to thrombin-anti-thrombin complexes have been reported in sepsis patients. The level of terminal complement proteins from C5 to C9 in plasma is normally about 500nM compared with the level of Vitronectin and clusterin about 4500 nM.

Vitronectin has been shown to be an integral constituent of the soluble C5b-9 (sC5b-9) also referred to as soluble membrane complex (sMAC), which is formed by Vitronectin and late components of complement proteins. On the cell membrane, activity of MAC is regulated by CD59, while in plasma, Vitronectin regulates the activity of MAC rendering it inactive.

Proceptor™ (CR-004) ELISA kit measures the Vitronectin bound to sC5b-9 complex. Our initial data has demonstrated two to three fold higher concentration of Vitronectin in CIC complex when compared to sMAC in the plasma.

**ASSAY PRINCIPAL**

The assay is based on unique capture of sC5b-9. The Vitronectin bound to sC5b-9 is measured thereafter using Vitronectin specific detection. The samples at appropriate concentration are pipetted into the wells. After washing away any unbound material, an antibody to Vitronectin is added to all the wells and then allowed to interact with the Vitronectin bound to the sC5b-9. Plates are washed to remove the unbound anti-Vitronectin antibodies. The wells are then filled with secondary antibody-enzyme reagent specific for the anti-Vitronectin antibody. After incubation a substrate (TMB) solution is added to the wells. Intensity of the color developed is proportionate to the amount of Vitronectin bound to the sC5b-9.

**REAGENTS**

**Receptor Microplate:** Polystyrene microtiter plate (12X8 wells) coated with antibodies to capture sC5b-9. Rows A1-H1 and A2-H2 are coated with varying concentration of purified Vitronectin protein. The standards are coated from 1.56 to 200 ng/ml in doubling dilution.

**Anti-Vitronectin Antibodies (7000103) (Rabbit):** One bottle containing 1.2 ml (10X) of anti-Vitronectin antibody (purified globulin fraction) with stabilization solution and preservative. Dilute with 1X wash buffer before use.

**HRP Conjugate (7000100)(Goat):** One bottle of 12 ml of secondary antibody against Vitronectin antibody. Purified immunoglobulin fraction conjugated to Horseradish peroxidase (HRP).

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

**Stop Solution (1000801):** One bottle 7ml 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 of collection. It is advised to assay immediately.

**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 Vitronectin.

SAMPLE PREPARATION
Serum or plasma is diluted 1:20 to 1:40 using 1X wash buffer. For samples having higher concentration of Vitronectin 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 (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 samples with 1X wash buffer. 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 sealer.
3. Wash the wells in the rest of the plate with 200 µl of 1X wash buffer for two to three minutes.
4. Pipette 100 µl of sample per well. It is recommended to run the assay in duplicate wells.
5. Incubate the plate at room temperature for ninety minutes preferably with gentle rotation.
6. Wash 4-times with 300 µl of wash buffer using a squirt bottle. Allow buffer to stay in the wells for 2 minutes between each wash.
7. Add 100 µl of anti-Vitronectin antibodies (diluted to 1X with wash buffer) to each well and incubate the plates 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 another 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 standard values against the known concentration and using the linear equation calculate the sample values (y = a + bX). Use the portion of the curve that is linear 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 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 Vitronectin

<table>
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<tr>
<th>Conc. ng/ml</th>
<th>OD 450 nm</th>
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<tbody>
<tr>
<td>1.5625</td>
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<tr>
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<tr>
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Slope 0.0194
Intercept 0.0715

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
Expiry Date:
Batch No.:
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