



## User's Manual

# Human Volociximab ELISA Kit

**REF** DEIASL511

**$\Sigma$**  96T

**RUO**

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

---

### Creative Diagnostics

 Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

 Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)  Fax: 1-631-938-8221

 Email: [info@creative-diagnostics.com](mailto:info@creative-diagnostics.com)  Web: [www.creative-diagnostics.com](http://www.creative-diagnostics.com)

---

## PRODUCT INFORMATION

### Intended Use

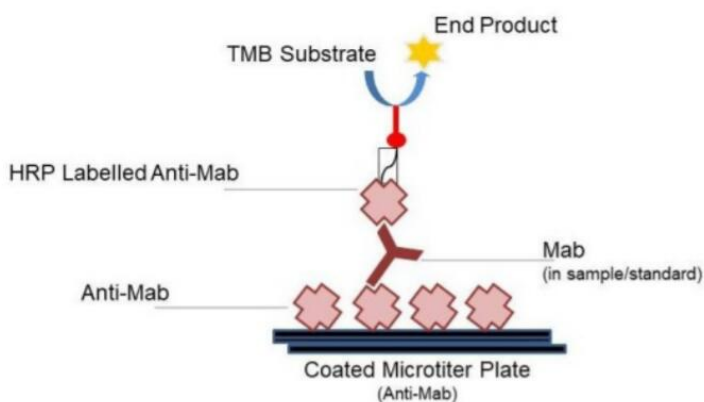
The Volociximab ELISA Kit is used as an analytical tool for quantitative determination of Volociximab in serum, plasma and cell culture supernatant.

### General Description

Volociximab is a high-affinity, chimeric monoclonal antibody that specifically binds to  $\alpha 5\beta 1$  integrin, a cell-surface receptor for fibronectin. Volociximab blocks the binding of  $\alpha 5\beta 1$  to fibronectin, thereby inhibiting a pivotal interaction required for angiogenesis.

### Principles of Testing

The method employs the quantitative sandwich enzyme immunoassay technique. Antibodies to Volociximab are pre-coated onto microwells. Samples and standards are pipetted into microwells and human Volociximab present in the sample are bound by the capture antibody. Then, a HRP (horseradish peroxidase) conjugated anti-Volociximab antibody is pipetted and incubated. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Volociximab in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.



### Reagents And Materials Provided

1. Anti-Volociximab Coated Microtiter Plate (12x8 wells) – 1 no
2. Volociximab Standard
3. Anti-Volociximab:HRP Conjugate – 12 ml
4. Sample Diluent – 50 ml
5. Wash Buffer (20X) – 25 ml
6. TMB Substrate – 12 ml
7. Stop Solution – 12 ml

## 8. Instruction Manual

### Materials Required But Not Supplied

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

### Storage

1. All reagents should be stored at 2°C to 8°C for stability.
2. All the reagents and wash solutions should be used within 12 months from manufacturing date.
3. Before using, bring all components to room temperature (18-25°C). Upon assay completion ensure all components of the kit are returned to appropriate storage conditions.
4. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

### Specimen Collection And Preparation

Blood is taken by venipuncture. Serum is separated after clotting by centrifugation. Plasma can be used, too. Lipaemic, hemolytic or contaminated samples should not be run. Repeated freezing and thawing should be avoided. If samples are to be used for several assays, initially aliquot samples and keep at - 20°C.

For Cell Culture Supernatant – If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

Preparation Before Use:

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently in order to ensure homogeneity.

Test Sample preparation –

- Serum Samples have to be diluted 1:100 (v/v), e.g. for 1:100 (1 ul sample + 99 ul sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

- Plasma Samples have to be diluted 1:1000 (v/v), e.g. for 1:1000 (1 ul sample + 999 ul sample diluent) prior to assay. The samples may be kept at 2 - 8°C for up to three days. Long-term storage requires -20°C.

### Reagent Preparation

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.

3. To make Wash Buffer (1X); dilute 25 ml of 20X Wash Buffer in 475 ml of DI water.

## Assay Procedure

1. It is strongly recommended that all Controls and Samples be run in duplicates. A standard curve is required for each assay. All steps must be performed at 37°C.
2. Add 100 µl of Standards or Samples into the respective wells.
3. Cover the plate and incubate for 60 minutes at 37°C.
4. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
5. Pipette without delay in the same order 100 µl of Anti-Volociximab: HRP Conjugate into each well.
6. Cover the plate and incubate for 60 minutes at 37°C.
7. Aspirate and wash plate 4 times with Wash Buffer (1X) and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
8. Add 100 µl of TMB Substrate in each well.
9. Incubate the plate at 37°C for 30 minutes in dark. DO NOT SHAKE or else it may result in higher backgrounds and worse precision. Positive wells should turn bluish in color.
10. Pipette out 100 µl of Stop Solution. Wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a microplate reader.

## Quality Control

It is recommended that for each laboratory assay appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

## Calculation

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using graph paper, plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Volociximab concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the Volociximab Concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a cubic spline curve-fit or a polynomial curve (2nd order) is best recommended for automated results.

Note:

It is recommended to repeat the assay at a different dilution factor in the following cases:

- If the sample absorbance value is below the first standard.
- If the absorbance value is equivalent or higher than the highest standard.

## Precautions

1. This kit is for Research Use only. Follow the working instructions carefully.
2. The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
3. Do not use or mix reagents from different lots.
4. Do not use reagents from other manufacturers.
5. Avoid time shift during pipetting of reagents.
6. All reagents should be kept in the original shipping container.
7. Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
8. Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
9. Since the kit contains potentially hazardous materials, the following precautions should be observed
  - Do not smoke, eat or drink while handling kit material
  - Always use protective gloves - Never pipette material by mouth
  - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
10. In any case GLP should be applied with all general and individual regulations to the use of this kit.

