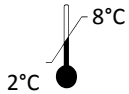
コスモ・バイオ株式会社

**BioMedica Diagnostics**
500 West Avenue
Stamford, CT 06902 USA
1.902.798.5105
www.biomedicadiagnostics.com

# IMUBIND® Factor VIIa ELISA

**REF 827**


## INTENDED USE

The IMUBIND® Factor VIIa ELISA is an enzyme-linked immunoassay for the quantitation of activated human factor VII (FVIIa) in plasma as well as in cell culture supernatants. This ELISA detects FVIIa as well as FVIIa complexed with Tissue Factor (TF/fVIIa). The assay is limited to research use only. It is not for use in diagnostic procedures.

## EXPLANATION OF THE TEST

Factor VII (FVII) is the first zymogen of the extrinsic pathway of blood coagulation. Activation of FVII occurs via cleavage of the proenzyme by proteases (e.g. factors IXa, Xa, XIIa and thrombin). Factor VII is also subject to auto-activation by Factor VIIa (FVIIa). The FVIIa molecule is the result of enzymatic cleavage at the Arg152-Ile153 bond. It consists of a 36,000 Dalton heavy chain and a 22,000 Dalton light chain held together by a disulfide bond. When FVIIa complexes with Tissue Factor, an enhanced enzymatic complex is formed that rapidly promotes coagulation. Tissue Factor Pathway Inhibitor (TFPI) negatively regulates the activity of the TF/FVIIa complex.

FVIIa levels in plasma are approximately 1% of FVII (≈5 ng/mL).

## PRINCIPLE OF THE PROCEDURE

The IMUBIND FVIIa ELISA employs a biotinylated enzyme inhibitor of Factor VIIa and an anti-FVII/FVIIa monoclonal antibody as the capture antibody. Diluted plasma samples or supernatants containing FVIIa are incubated with the biotinylated inhibitor, which covalently attaches to the FVIIa but not to FVII. The samples are added to microwells precoated with the FVIIa capture antibody. FVIIa is detected by binding of the streptavidin conjugated horseradish peroxidase (HRP) conjugate to the immunocaptured FVIIa/biotinylated inhibitor complex. The addition of TMB substrate and its subsequent reaction with HRP provides a blue color. Sensitivity is increased by addition of a 0.5N sulfuric acid stop solution, yielding a yellow color. FVIIa levels are determined by measuring sample solution absorbance at 450 nm and comparison against those of a standard curve developed using known amounts of fVIIa.

**REAGENTS** (sufficient for 40 plasma samples, assayed in duplicate)

96 MAb Anti-Human FVII/FVIIa coated microwells with acetate cover sheet
96 microwell plate, uncoated
2 vials of FVIIa Standard, 200 ng/mL (lyophilized)
1 vial of FVII Deficient Plasma, 300 µL (lyophilized)
1 vial of FVIIa Inhibitor, biotinylated, 160 µL (lyophilized concentrate)
1 vial of Assay Diluent, 22 mL (lyophilized)
1 vial of Reference Plasma, 300 µL (lyophilized)
1 vial of Stabilizer, 3.5 mL (lyophilized)
1 vial of Enzyme Conjugate, Streptavidin-horseradish peroxidase, 120 µL
1 vial of Substrate, TMB, 11 mL
1 packet of Wash Buffer, PBS with 0.05% Tween 20, 1 Liter (lyophilized)



827_D©BMD20171121

## WARNINGS AND PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method can provide complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious human specimen.

Reagents supplied in this kit contain sodium azide ($NaN_3$), which may form explosive metallic azides upon reaction with copper and lead plumbing. Flush with large volumes of water during disposal to prevent azide build-up.

FOR RESEARCH USE ONLY. Do not use kit components beyond the printed expiry date. Do not mix reagents from different kit lots. Avoid microbial contamination of kit components. Do not mouth pipette or ingest any of the reagents. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.

| | | | |
|---|---|---|---|
| Assay Diluent | Danger (US)<br>Warning (EU) |  | H315, H319, H334, H317, P264, P280, P302 + P352, P332 + P313, P305 + P351 + P338, P337 + P313 |
| Stabilizer | Danger (US)<br>Warning (EU) |  | H315, H319, H334, H317, P264, P280, P302 + P352, P332 + P313, P305 + P351 + P338, P337 + P313 |
| Enzyme Conjugate | Warning |  | CONT 2-methyl-4-isothiazol-3 one<br>H317, P261, P280, P302 + P352, P333 + P313 |
| Wash buffer | Warning |  | H319, P264, P280, P305 + P351 + P338, P337 + P313 |

**Hazard Statements:**
- H315 Causes skin irritation.
- H317 May cause an allergic skin reaction.
- H319 Causes serious eye irritation.
- H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

**Precautionary statements:**
- P261 Avoid breathing mist or vapor.
- P264 Wash thoroughly after handling.
- P280 Wear eye protection/face protection. Wear protective gloves.
- P302 + P352 If on skin: Wash with plenty of water.
- P305 + P351 + P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- P332 + P313 If skin irritation occurs: Get medical advice/attention.
- P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
- P337 + P313 If eye irritation persists: Get medical advice/attention.



## REAGENT PREPARATION AND STORAGE

### Procedural Notes

1. FVIIa Standard should be used within 30 minutes after reconstitution. Do **not** prepare standards in advance**.** Use only freshly reconstituted FVIIa standards for each assay. Two vials of lyophilized FVIIa Standard are provided for performance of two assays at separate times.
2. All other reagents may be stored at -20°C after reconstitution.

### A. FVIIa Standard

Reconstitute to the volume indicated on the vial label with cold (2°-8°C) filtered deionized water. Allow the vial to stand on ice for 2-3 minutes. Vortex the vial to achieve adequate mixing.

### B. Stabilizer

Reconstitute with 3.5 mL of cold (2°-8°C) filtered deionized water to the vial.

### C. FVIIa Inhibitor

Prior to reconstitution, centrifuge the tube to collect the FVIIa Inhibitor at the bottom, of the tube. Reconstitute with 160 µL of 1 mM HCl. Vortex vigorously to assure all solids are dissolved and place the vial on ice until use.

### D. Assay Diluent

Reconstitute with 22 mL of filtered deionized water and mix well.

### E. Reference Plasma

Reconstitute with 0.3 mL of cold (2°-8°C) filtered deionized water. Vortex the vial to achieve adequate mixing.

### F. FVII Deficient Plasma

Reconstitute with 0.3 mL of cold (2°-8°C) filtered deionized water.

### G. Wash Buffer

Dissolve the contents of the Wash Buffer packet in 1 liter of filtered deionized $H_2O$.

Store unused micro-test strip-wells and intact (unreconstituted) reagents at 2 - 8°C until the expiration dates indicated on label.

Store reconstituted reagents (except FVIIa Standard) at -20°C for up to one month. Remember to aliquot and freeze reconstituted plasmas **immediately**.



827_D©BMD20171121

## SPECIMEN COLLECTION AND PREPARATION

The IMUBIND Factor VIIa ELISA is for use with solutions containing purified FVIIa, cell supernatants and citrated or EDTA collected plasma. See "Collection, Transport and Processing of Blood Specimens for Testing Plasma-Based Coagulation Assays and Molecular Hemostasis Assays; Approved Guideline - Fifth Edition", CLSI document H21-A5, Vol. 28, No. 5, 2008. Collection of plasma for testing FVIIa levels should be done as follows:

1. Collect 9 parts of blood into 1 part of 3.8% (0.129M) trisodium citrate anticoagulant solution or 99 parts of blood into 1 part 0.5M EDTA.
2. Centrifuge the blood sample at 2,500 rpm for 15 minutes.
3. Plasma may be stored for up to 4 hours at room temperature (20° - 25°C), up to 8 hours at 2° - 8°C, or up to 1 month at -20°C or colder.
4. Frozen plasma should be thawed at 37°C for 15 minutes before testing.

## PROCEDURE

### Materials Provided – See Reagents

### Materials Required But Not Provided

0.22 µm filtered deionized water
50-200 µL eight channel multi-pipette, 10-200 µL single pipette
Microwell plate reader at 450 nm
0.5N $H_2SO_4$ (use caution when handling)
1 mM HCl (use caution when handling)

### A. Preparation of FVIIa Standards and Plasma Samples

**Procedural Note:** Use the uncoated 96 microwell plate for preparation of all standards and plasmas. See Schematic, in Table 1.

1. To wells A1 and A2, add 125 µL of FVIIa Standard and 65 µL of Stabilizer.
2. To wells B1 - F1 and B2 - F2, add to each: 50 µL of Assay Diluent, 12.5 µL of FVII Deficient Plasma, and 32.5 µL of Stabilizer.
3. To wells G1 and G2, add 37.5 µL of Assay Diluent, 12.5 µL of Reference Plasma, 12.5 µL of FVII Deficient Plasma and 32.5 µL of Stabilizer.
4. To wells H1 and H2, add 50 µL of Assay Diluent, 12.5 µL of FVII Deficient Plasma and 32.5 µL of Stabilizer.
5. To the remaining available wells (A3-A12, B3-B12, C3-C12, etc.), add 50 µL of Assay Diluent, 12.5 µL of plasma sample and 32.5 µL of Stabilizer.



6. Prepare working strength FVIIa Inhibitor by diluting 1:20 with Assay Diluent. Prepare an amount sufficient for the number of desired tests. Discard unused working strength inhibitor.
7. Add FVIIa Inhibitor to the wells as follows:
   a. Add 60 µL to wells A1 and A2.
   b. Add 30 µL to all remaining wells including wells containing plasma samples.
8. Incubate the plate for 10 minutes at 2° - 8°C.

**Table 1: Schematic of Sample Preparation in Uncoated Microwell Plate**

| | Wells A1,A2 | Wells B1-F1, B2-F2 | Wells G1,G2 | Wells H1,H2 | Sample Wells |
|---|---|---|---|---|---|
| Diluent | ------ | 50 µL | 37.5 µL | 50 µL | 50 µL |
| FVIIa Standard | 125 µL | ------ | ------ | ------ | ------ |
| Reference Plasma | ------ | ------ | 12.5 µL | ------ | ------ |
| Test Plasma Samples | ------ | ------ | ------ | ------ | 12.5 µL |
| FVII Deficient Plasma | ------ | 12.5 µL | 12.5 µL | 12.5 µL | ------ |
| Stabilizer | 65 µL | 32.5 µL | 32.5 µL | 32.5 µL | 32.5 µL |
| Inhibitor | 60 µL | 30 µL | 30 µL | 30 µL | 30 µL |

9. After the 10-minute incubation period, generate FVIIa standard levels by serial dilution. The FVIIa standard in wells A1 and A2 is now at 100 ng/mL (1:1 dilution of the 200 ng/mL stock). Serially dilute the 100 ng/mL FVIIa standard to make 5 additional FVIIa standards of 50, 25, 12.5, 6.25 and 3.12 ng/mL, respectively, as follows. Transfer 125 µL from well A1 into well B1 and well A2 into well B2 and mix. Repeat this transfer from well B1 to well C1 and B2 to well C2. Repeat through wells F1 and F2. NOTE: The Assay Diluent in wells H1 and H2 serves as the 0 ng/mL standard.

### B. Assay Procedure

1. Transfer 100 µL of the FVIIa standards and the plasmas from the wells of the uncoated 96 microwell plate to the corresponding FVIIa antibody coated microwell location.
2. Cover the strips and incubate for 1 hour at 2° - 8°C.
3. Wash wells 4 times with Wash Buffer.

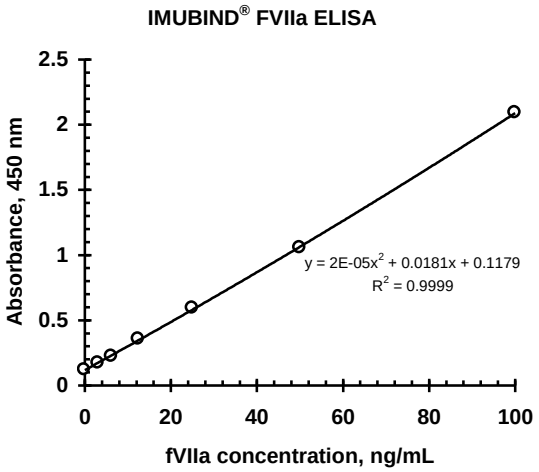


827_D©BMD20171121

4. Prepare working strength Enzyme Conjugate by diluting 1:200 with Assay Diluent. For running all 96 wells at one time, dilute 60 µL of conjugate up to 12 mL in Assay Diluent (10 µL added to 2 mL of Assay Diluent for each 16 well strip used). Add 100 µL of diluted enzyme conjugate to each well, cover and incubate for 30 minutes at room temperature.
5. Wash wells 4 times with Wash Buffer.
6. Add 100 µL of Substrate solution to each well, cover and incubate for 20 minutes at room temperature. A blue color will develop.
7. Stop the enzymatic reaction by adding 50 µL of 0.5N $H_2SO_4$. Tap the sides of the strip-wells to ensure even distribution of the $H_2SO_4$. The solution color will turn yellow. Read the absorbance on a microwell plate reader at a wavelength of 450 nm within 30 minutes.

## RESULTS

The standard curve is constructed by plotting the mean absorbance value for each FVIIa standard versus the corresponding concentration of FVIIa in ng/mL. The average absorbance of the 0 ng/mL standard may be deducted from the values of the standards, reference plasma and test plasmas. Interpolate the FVIIa concentrations for the plasma samples directly from the standard curve. A standard curve should be generated each time the assay is performed. The following curve is for demonstration purposes only.




## CALCULATIONS

Although plasma samples are run neat in the assay, the assay procedure creates a 1:10 dilution factor. After converting the average absorbance of the sample to a FVIIa concentration using the standard curve, multiply this concentration by a factor of ten to obtain the actual FVIIa concentration of the plasma sample.



If the absorbance values of samples fall outside the range of the standard curve, dilute the sample with Assay Diluent and repeat the assay. After determining the FVIIa concentration in the diluted sample, multiply this concentration by the dilution factor used to calculate the FVIIa concentration of the original sample.

## EXPECTED VALUES

FVIIa in normal plasmas is approximately 5 ng/mL.

## PERFORMANCE CHARACTERISTICS

### Analytical Specificity

This assay recognizes native and recombinant human FVIIa and FVIIa/TF complexes.[5] No significant amount of FVII is detected in the assay. FVII does not autoactivate to FVIIa during performance of this assay.

### Analytical Sensitivity

The lower limit of detection was determined by adding 2 standard deviations to the mean OD value (n = 15) for the 0 ng/mL standard and calculating the corresponding concentration from the standard curve.

Those persons wishing to measure FVIIa levels outside the range of the assay should contact the Technical Services Department at BioMedica Diagnostics.

## REFERENCES


1. Morrissey, J. H., *et al. Blood* 1993, **81**: 734.
2. Fiore, M. M., *et al. Journal of Biological Chemistry* 1994, **269**: 143.
3. Pedersen, A. H., *et al. Biochemistry* 1989, **28**: 9331.
4. Persson, E., *et al. Journal of Biological Chemistry* 1997, **272**: 19919.
5. Goudemand, J, *et al. Blood Coagulation and Fibrinolysis* 2003, **14**: 505-511.


## DEFINITION OF SYMBOLS

| | | | |
|---|---|---|---|
| | Consult instructions for use | REF | Catalog Number |
| | Manufacturer | LOT | Lot Number |
| | Refer to SDS, Safety Data Sheet | | Expiration Date |
| | Temperature Limitation | Σn | Contains sufficient for <n> tests |
| CONT | Contains... | | |



827_D©BMD20171121