# Human Factor VIIa Activity Assay Kit

BGT-KAT-01000

96Wells

**RUO**

Enzyme Immunoassay for the Quantitative Determination of Factor VIIa in human serum and plasma

**Introduction:**

Factor VII (FVII) is a vitamin K-dependent plasma glycoprotein that is synthesized in the liver and circulates in blood as a single-chain inactive zymogen with a molecular mass of 50 kDa (1). Upon tissue damage and vascular injury, the cell surface receptor and cofactor tissue factor (TF) binds and allosterically activates FVII to its active form, FVIIa. The TF/FVIIa complex catalyzes the conversion of both factor IX to factor IXa and factor X to factor Xa to initiate coagulation via the extrinsic pathway (2-3). Very low levels of FVII are associated with severe coagulation disorders (4). Elevated plasma levels of FVII coagulant activity constitute an independent risk factor for fatal outcomes of coronary heart disease in middle-aged men (5).

**Principle:**

The Human Factor VIIa Activity Assay Kit is developed to determine FVIIa activity in human plasma, serum, and cell culture samples. This kit is also validated for use with bovine, equine, monkey, mouse, rat, swine, and rabbit samples. This assay couples immunofunctional and indirect amidolytic function. A polyclonal antibody specific for human FVIIa has been pre-coated onto a 96-well microplate with removable strips, and FVIIa is bound to the immobilized antibody. The assay measures the ability of FVIIa to activate factor X (FX) to factor Xa (FXa). The amidolytic activity of the FVIIa is quantitated by the amount of FXa produced using a highly specific FXa substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA at 405 nm is directly proportional to the FVIIa enzymatic activity.

**Materials Provided:**

| Part | Description | Qty |
|---|---|---|
| Human Factor VIIa Microplate | A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human FVIIa. | 1 x 96 wells |
| Human Factor VIIa Standard | Calibrated against WHO 2nd International Standard | 12000 ng, lyophilized |
| EIA Diluent Concentrate (10x) | A 10-fold concentrated buffered protein base | 20 ml |
| Wash Buffer Concentrate (20x) | A 20-fold concentrated buffered surfactant | 30 ml |
| Assay Diluent (1x) | Buffered protein base | 20 ml |
| Human FX | Lyophilized. | 2 vials |
| FXa Substrate | Lyophilized | 2 vials |

**Materials to be provided by the End-User:**

1. Microplate reader capable of measuring absorbance at 405 nm
2. Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
3. Deionized or distilled reagent grade water
4. Incubator (37°C)

**Handling/Storage:**

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date
- Store Standard, FX, and FXa Substrate at -20°C.
- Store Microplate, Assay Diluent, EIA Diluent Concentrate (10x), and Wash Buffer Concentrate (20x) at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. For Research Use Only.

**Sample Collection, Preparation, and Storage:**

- **Plasma**: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum**: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatant:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If necessary, dilute samples into EIA Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

| **Guidelines for Dilutions of 100-fold or Greater**<br>(for reference only; please follow the insert for specific dilution suggested) | |
|---|---|
| **100x** | **10000x** |
| A) 4 µl sample : 396 µl buffer (100x)<br>= 100-fold dilution<br><br>Assuming the needed volume is less than or equal to 400 µl. | A) 4 µl sample : 396 µl buffer (100x)<br>B) 4 µl of A : 396 µl buffer (100x)<br>= 10000-fold dilution<br>Assuming the needed volume is less than or equal to 400 µl. |
| **100x** | **10000x** |
| A) 4 µl sample : 396 µl buffer (100x)<br>B) 24 µl of A : 216 µl buffer (10x)<br>= 1000-fold dilution<br><br>Assuming the needed volume is less than or equal to 240 µl. | A) 4 µl sample : 396 µl buffer (100x)<br>B) 4 µl of A : 396 µl buffer (100x)<br>C) 24 µl of B : 216 µl buffer (10x)<br>= 100000-fold dilution<br>Assuming the needed volume is less than or equal to 240 µl. |

**Preparation Before Use:**

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

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

- Bring all reagents to Room temperature before use.
- **EIA Diluent Concentrate (10x)**: Dilute the EIA Diluent Concentrate 10-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved. Store for up to 30 days at 2-8°C.
- To make Wash Buffer (1X); dilute 30 ml of 20X Wash Buffer in 570 ml of DI water.
- **Human FX**: Add 1.1 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- **FXa Substrate**: Add 1.1 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and used within 10 days.
- **Wash Buffer Concentrate (20x):** Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

- **Human Factor VIIa Standard**: Reconstitute the Human Factor VIIa Standard (12000 ng, 720 IU) with 1.5 ml of EIA Diluent to generate an 8000 ng/ml (480 IU/ml) standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (8000 ng/ml) 4-fold with EIA Diluent to produce 2000, 500, 125, 31.25, and 7.813 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

| Standard Point | Dilution Particulars | [FVIIa] (ng/ml) | [FVIIa] (IU/ml) |
|---|---|---|---|
| P1 | 1 part Standard (8000 ng/ml) + 3 parts EIA Diluent | 2000 | 120 |
| P2 | 1 part P1 + 3 parts EIA Diluent | 500 | 30 |
| P3 | 1 part P2 + 3 parts EIA Diluent | 125 | 7.5 |
| P4 | 1 part P3 + 3 parts EIA Diluent | 31.25 | 1.875 |
| P5 | 1 part P4 + 3 parts EIA Diluent | 7.813 | 0.469 |
| P6 | EIA Diluent | 0.0 | 0.0 |

**Assay Procedure:**

1. Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use.

2. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.

3. **The assay is incubated at 2-8°C for binding of standard and samples and at 37°C for chromogenic activity.**

4. Add 100 µl of Human Factor VIIa Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate at 2-8°C overnight or for at least 12 hours. Prepare Assay Mix Reagent calculations prior to the next step.

5. **Prepare Human FX and FXa Substrate prior to washing the microplate.** Allow the vials to sit for 10 minutes with gentle agitation prior to use.

6. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.

7. Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the number of wells in the assay (n) plus one well.

| Assay Mix Reagent | n = 1 well |
|---|---|
| Assay Diluent | 100 µl |
| Human FX | 20 µl |

8. Add 120 µl of Assay Mix to each well, and immediately add 20 µl of FXa Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm for a zero minute background reading. Cover wells with a sealing tape and incubate at 37°C in a humid incubator to avoid evaporation.

9. Read the absorbance (405 nm) at 20 hours and continue reading every hour up to 28 hours. Cover wells with a sealing tape and incubate at 37°C after each reading.

**Data Analysis**

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve from the optimal reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance (OD) or change in absorbance per minute (ΔA/min) on the y-axis after subtracting the background. The best fit line can be determined by regression analysis of the 4-parameter curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

**Typical Data**

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

| Standard Point | ng/ml | Average OD |
|---|---|---|
| P1 | 2000 | 0.424 |
| P2 | 500 | 0.331 |
| P3 | 125 | 0.181 |
| P4 | 31.25 | 0.098 |
| P5 | 7.813 | 0.074 |
| P6 | 0.0 | 0.067 |

**Performance Characteristics**

- **Kit standard has been calibrated against WHO International Standard**.
- The minimum detectable dose of human FVIIa as calculated by 2SD from the mean of a zero standard was established to be 7.1 ng/ml.

**Safety Precautions:**

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- 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.
- 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.
- 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.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.