ZYMUTEST Factor VII

 # RK036A

(Complete ELISA kit for Factor VII Antigen)

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

The ZYMUTEST Factor VII kit is a two-site immuno-assay for measuring human Factor VII (FVII) Antigen in plasma, or in any fluid where FVII can be present.
This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

First, the diluted tested sample is introduced into the microwells coated with a rabbit polyclonal antibody specific for FVII. Then after, the immunoconjugate, which is a rabbit polyclonal antibody specific for FVII coupled to horse radish peroxidase (HRP), is introduced, and the immunological reaction starts. When present, FVII binds onto the polyclonal antibody coated solid phase, and fixes the polyclonal antibody coupled to HRP through free epitopes. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), in presence of hydrogen peroxide (H_2O_2), is introduced and a blue colour develops. When the reaction is stopped with Sulfuric Acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human FVII in the tested sample.

TEST SAMPLE:

- Trisodium Citrate (or Na_2 EDTA) anticoagulated human plasma.
- Any biological fluid where FVII or FVII(a) protein concentration must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with a rabbit polyclonal antibody specific for human FVII, then stabilised; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD:** 2 vials containing 50ml of **Sample Diluent**, ready to use.
3. **Cal:** 3 vials of **Plasma Factor VII Calibrator**, lyophilised. When restored with **2 ml** of sample diluent, a ready to use plasma calibrator (**already diluted 1:20**) containing a concentration "**C"** (expressed in %) of human FVII is obtained. This concentration (usually in the range 130-160% according to the lot, established by reference to the NIBSC standard) is accurately determined for each lot and indicated on the flyer provided in the kit.
4. **CI:** 1 vial containing **0.5 ml** of lyophilised **Plasma Control I High** (human plasma).
5. **CII**: 1 vial containing **0.5 ml** of lyophilised **Plasma Control II Low** (human plasma).

Note *:* The FVII concentrations and acceptance ranges for controls can vary from lot to lot, and are indicated on the flyer provided in the kit.

6. **IC:** 3 vials of **Anti-(h)-FVII-HRP immunoconjugate**, rabbit polyclonal antibody specific for human FVII, coupled to Horse-Radish-Peroxidase (HRP), lyophilised.
7. **CD:** 1 vial of 25 ml of **Conjugate Diluent**, ready to use.
8. **WS:** 1 vial of 50 ml of 20 fold concentrated **Wash Solution**.
9. **TMB:** 1 vial of 25 ml peroxidase substrate: **3,3',5,5' – Tetramethylbenzidine** containing hydrogen peroxide. Ready to use.
10. **SA:** 1 vial of 6 ml of **0.45M Sulfuric acid** (Stop solution). Ready to use.

Note**:** Use only components from a same kit lot. Do not mix components from different lots, when running the assay.

REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED:

- **8-channel** or repeating **pipette** allowing dispensing 50-300 µl.
- **1-channel pipettes** at variable volumes from 0 to 20 µl, 20 to 200 µl and 200 to 1000 µl.
- **Micro ELISA plate** washing equipment and shaker.
- Micro ELISA plate **reader** with a wavelength set up at 450 nm.
- Distilled water.

REAGENTS PREPARATION, STORAGE AND STABILITY:

In their original packaging box, before use, when stored at 2-8°C, the unopened reagents are stable until the expiration date printed on the box.

Note**:** The stability studies for 3 weeks at 30°C show that the reagents can be shipped at room temperature for a short period without any damage.

1. **Micro ELISA plate:** open the plastic pouch and take off the required amount of 8 well strips for the test series. When out of the pouch, the strips must be used within 30 minutes. Unused strips can be stored at **2-8°C** for **4 weeks** in their original aluminium pouch, in presence of the desiccant, hermetically closed and protected from any moisture, and stored in the provided microplate storage bag (minigrip).
2. **Sample Diluent:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
3. **Plasma Factor VII Calibrator:** Restore each vial with **2 ml** of sample diluent in order to obtain a plasma containing a FVII concentration **"C"** (in %), **already diluted 1:20** (twenty fold), ready for use. This solution is stable for at least **24 hours at room temperature** or **72 hours at 2-8°C.**
4. **Plasma Control I** (human plasma, high): restore with **0.5 ml** distilled water.
5. **Plasma Control II** (human plasma, low): restore with **0.5 ml** distilled water.

Note: when restored, Factor VII controls are stable for at least **24 hours** at room temperature, **72 hours at 2-8°C** or **2 months** frozen **at –20°C** or below.

Warning: Plasma controls I and II (4&5) and calibrator (3) are prepared with normal human plasma. This latter was tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. However, no assay may warrant the total absence of infectious agents. **Bovine Serum Albumin (BSA**), included in some reagents (Cal, CI, CII, IC, CD, SD), was prepared from bovine plasma, which was tested for the absence of infectious agents, and collected from animals free from BSE. However, no assay may warrant the total absence of infectious agents. Any product of biological origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti-(h)-FVII-HRP immunoconjugate**: each vial must be restored with **7.5 ml of Conjugate Diluent**. Let the pellet completely dissolve before use, and shake the vial gently in order to homogenize the contents. The restored conjugate is stable for at least **24 hours** at room temperature or for at least **4 weeks at 2-8°C**.
7. **Conjugate Diluent**: It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8 °C**, and provided that any bacterial contamination is avoided during use. This reagent contains 0.05% Kathon CG.
8. **Wash Solution**: Incubate the vial for 15-30 minutes in a water bath **at 37°C** until complete dissolution of solids, when present. Shake the vial and dilute the amount required 1:20 in distilled water (the 50 ml contained in the vial allow preparing 1 liter of Wash Solution). The Wash Solution must be stored at **2-8°C** in its original vial and used within **4 weeks** following opening. The diluted Wash Solution must be used within **7 days**, when protected from any contamination and stored at **2-8°C**. This reagent contains 0.05% Kathon CG.
9. **TMB substrate:** It is ready to use. When open, it can be used for **4 weeks**, stored at **2-8°C**, and provided that any bacterial contamination is avoided during use.
10. **Stop solution:** It is ready to use.

Cautions*:* Sulfuric acid, although diluted to 0.45M is caustic. As for any similar chemical, handle Sulfuric acid with great care. Avoid any skin and eye contact. Wear protection glasses and gloves when handling.

Note**:** Bring the kit at room temperature, at least 30 min. before use. Store the unused reagents at 2-8°C.

TESTED SPECIMEN:

Sample: Human citrated plasma or FVII or FVII(a) concentrates.
Collection and preparation: Blood (9 vol.) must be collected on 0.109M (or 0.129M) citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g.
Stability/Storage: citrated plasma should be tested within **8 hours** or stored frozen at –20°C or colder for up to 6 months, and thawed for 15 min. at 37°C just before use.

Note: Refer to GEHT or CLSI recommendations for further instructions on specimen collection, handling and storage. Discard any plasma presenting an unusual aspect (haemolysed, lipaemic aspect….).

EDTA collected human plasma may also be used. Conditions of storage are the same than those for citrated plasma.

PROCEDURE:

Tested plasma or sample or controls:

The sample must be tested **diluted twenty fold (1:20)** in the Sample Diluent. For expected FVII concentrations higher than "**C**" (in %), plasma or samples must be tested at a higher dilution, i.e., **1:40 (D=40), or more.** For low FVII concentrations, lower dilutions can be used (e.g., **1:10**, i.e., **D=10**).

Plasma **Controls I and II** must be tested diluted **twenty fold (1:20)**, with Sample Diluent.

Calibration:

Using the **Plasma FVII Calibrator,** with a FVII concentration "**C**" (corresponding to a plasma already diluted **1:20**) provided in the kit, prepare the following standard solutions.

Factor VII concentration (%)	C	C/2	C/4	C/10	C/20	0
Vol. of Plasma FVII calibrator at C	1 ml	0.5 ml	0.25 ml	0.1 ml	0.05 ml	0 ml
Vol. of Sample diluent	0 ml	0.5 ml	0.75 ml	0.9 ml	0.95 ml	1 ml

Mix gently for a complete homogenization.

The standard dilutions are stable for **4 hours** at room temperature.

Assay procedure:

Remove the required number of 8-well strips from the aluminium pouch, for the series of measures to be performed. Then put the strips in the frame provided. In the different wells of the micro ELISA plate, introduce the reagents and perform the various assay steps as indicated on the following table:

Reagent	Volume	Procedure
FVII calibrator or diluted tested sample or controls or sample diluent (blank)	**50 µl**	Introduce **immediately** the standard solutions or the tested samples in the corresponding micro ELISA plate well
Incubate for 15 minutes at room temperature (18-25°C) (a)		
Conjugate anti (h)-FVII-HRP. (Restored with 7.5 ml of conjugate Diluent)	**200 µl**	Introduce the Anti-(h)-FVII- HRP immunoconjugate in the micro ELISA plate wells
Incubate for 1 hour at room temperature (18-25°C) (a) Then wash the plate :		
Wash Solution (20 fold diluted in distilled water before use)	**300 µl**	Proceed to 5 successive washings using the washing instrument. **(b)**
TMB/H_2O_2 Substrate	**200 µl**	Immediately after the washing, introduce the substrate into the wells. (b) ***Note :*** The substrate distribution, row by row, must be accurate and at exact time intervals **(a, c)**.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (a)		
0.45 M Sulfuric Acid (5)	**50 µl**	Following exactly the same time intervals than for the addition of substrate, stop the colour development by introducing the 0.45M sulfuric acid **(c).**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450)**. Subtract the blank value **(d)**.		

Note:

Distribute calibrators, controls and tested specimen as rapidly as possible, in order to obtain homogeneous immunological kinetics for FVII binding. A too long delay (>10 min) between the distribution of the first and the last wells may have incidence on immunological kinetics and produce wrong results (underestimated value for the last wells).

a. Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development.. An incubation temperature of 18-25°C must be respected. Results can be affected by a too high (>25°C) or too low (<18°C) temperature, and measured A450 could then be too high or too low. It has to be considered when analyzing the results. A450 values generated in the assay are susceptible to be significantly increased if shaking is used throughout the incubation steps.

b. Never let the plates empty between the addition of the reagents or following the washing step. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilised components. If necessary, keep the plate filled with Wash Solution and empty it just before the introduction of the next reagent. The washing instrument must be adjusted in order to wash the plates gently, and to avoid a too drastic emptying, which could lower plate reactivity.

c. For addition of the TMB substrate, the time interval between each row must be accurate and exactly determined. It must be the same when stopping the reaction with sulphuric acid.

d. For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used

EXPRESSION OF RESULTS:

- On a linear graph paper plot the **FVII concentration (%)** on abscissa and the corresponding absorbance (**A450**) on ordinates. Draw the calibration curve (best fit, or second order polynomial regression). Alternatively, a log-log curve can be used (use log-log graph paper).
- Users must construct their own calibration curve, obtained using their calibrator dilutions (**See model on the flyer**). From the curve obtained, deduce directly the FVII concentration for the tested sample when assayed at the standard dilution. For obtaining the FVII concentration in a sample tested at a higher or lower dilution, this value must be multiplied by **D:20** (e.g., x2 for a sample tested at the **1:40** dilution , where D=40, or x0.5 for a sample tested at the 1:10 dilution where D=10).
- For **controls I and II**, tested at the standard **1:20** dilution, concentrations are directly deduced from the calibration curve.

The calibration curve is valid when measured values for the controls are in compliance, within the defined acceptance range indicated on the flyer included in the kit.

- Alternatively, an ELISA software (i.e., Dynex, Biolise, etc.) can be used for the calculation of concentrations (select the best fit curve or a second order polynomial regression).

The results obtained should be for research purposes only and not used for patient diagnosis or treatment.

TWO STEP METHOD:

- The assay can also be performed with a two step method. All specimens are assayed at a 5-fold higher dilution. The assay dilution is then 1:100. The calibration curve is from **0 to C%**, the FVII Calibrator (Cal) being reconstituted with **2 ml** of Sample Diluent (**SD**) and **then diluted 1:5** (solution at C%, corresponding to a plasma already diluted **1:100**). Prepare calibration dilutions as for the one-step method but starting with the concentration "**C**" corresponding to a 1:5 dilution.
- The immunoconjugate (**IC**) must be reconstituted with **7.5 ml** of Conjugate Diluent (**CD**).
- Tested plasma and controls CI and CII must be assayed at a 100 fold (**1:100**) dilution or at higher dilutions in Sample Diluent (**SD**), if required.

-In each microwell, **200 µL** of the calibration solution are introduced (prepared from the calibrator **prediluted 1:5**) or 200µL of the 1:100 (or more) diluted tested plasma. Following a **1 hour** incubation at room temperature (18-25°C) and a washing step, **200 µl**/well of immunoconjugate (**IC**) are introduced. Following a new **1 hour** incubation at room temperature and a washing step, the colour development with TMB (**200 µl**/well) is allowed to develop for **5 min**, and is then stopped with **50 µl** of 0.45M sulfuric acid (**SA**). A450 is then measured. Washing and operating cautions, as well as results interpretation, are the same as recommended for the one step method. For samples and controls tested at the 1:100 dilution, concentrations are directly deduced from the calibration curve.

BIOCHEMISTRY:

Factor VII (FVII) is a vitamin-K dependent serine-protease, single chain glycoprotein, produced by the liver. Factor VII circulates as a zymogen in human plasma, at a concentration of about 0.5 µg/ml. It can be cleaved by thrombin, FIXa, FXa, FXIIa or FVIIa (autoactivation) to produce FVIIa. In the presence of calcium and phospholipids, activated FVII(a) forms a procoagulant complex with Tissue Factor, which converts Factor X and Factor IX in activated forms, able to generate thrombin and the fibrin clot. TFPI, a Künitz type inhibitor, blocks the activation of FX by FVII-Tissue factor complex, by forming a quaternary complex with Tissue Factor, Factor VII and FXa, inactive.

APPLICATIONS:

- Assay of FVII:Ag in human plasma samples.
- Assay of FVII:Ag in concentrates.
- Assay of plasma FVIIa, and of FVII or FVIIa in purified milieu.

ASSAY CHARACTERISTICS:

- Detection threshold ≤ 5%.
- Intra-assay CV: 3-5%.
- Inter-assay CV: 3-8%.
- No significant heparin interference up to 2 IU/ml.