ZYMUTEST Factor VII

Ref RK036A
96 tests

ELISA kit for Factor VII antigen

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

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INTENDED USE:

The ZYMUTEST Factor VII kit is a "sandwich" ELISA method for measuring Factor VII (FVII) antigen in human plasma, or in any biological medium where FVII is present.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Factor VII (FVII) is a vitamin-K dependent serine-protease, single chain glycoprotein, produced by the liver. 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 IX and X 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 an inactive quaternary complex with Tissue Factor, Factor VII and FXa.

PRINCIPLE:

First, the immunoconjugate, a rabbit polyclonal antibody specific for FVII coupled to horse radish peroxidase (HRP), is introduced into the microwells of ELISA plate coated with a polyclonal antibody specific for FVII. Then, the diluted tested sample is immediately introduced, and the immunological reaction starts. The FVII present in the specimen, binds onto the polyclonal antibody coated solid phase through one epitope, 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 in the microwells 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.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with a rabbit polyclonal antibody specific for human FVII, stabilised and packed in an aluminium pouch hermetically sealed in presence of a desiccant. Contains BSA.
2. **SD:** 2 vials containing 50 mL of **Sample Diluent**, ready to use. Contains BSA.
3. **CAL:** 3 vials of **FVII Calibrator (calibrator plasma)**, lyophilised. Each vial must be restored with **2 mL** of Sample Diluent (SD) to obtain a calibrator containing a concentration "**C"**, expressed in % of human FVII (**already diluted at 1:20**), precisely determine for each lot and indicated on the flyer provided with the kit. This concentration "C" is greater than or equal to 100%, according to the lot. This calibrator is related to the NIBSC international standard. Contains BSA.
4. **CI:** 1 vial containing **0.5 mL** of lyophilised **Plasma FVII Control I High** (human plasma). Contains BSA.
5. **CII:** 1 vial containing **0.5 mL** of lyophilised **Plasma FVII Control II Low** (human plasma). Contains BSA.
6. **IC:** 3 vials of **Anti-(h)-FVII-HRP immunoconjugate**, rabbit polyclonal antibody coupled to Horse-Radish-Peroxidase (HRP), lyophilised. Contains BSA.
7. **CD:** 1 vial of 25 mL of **Conjugate Diluent**, ready to use. Contains BSA.
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.

The exact concentration of controls and calibrator, and the acceptable interval concentration for the controls are indicated on the flyer provide in the kit. Concentrations vary from lot to lot. For the assay, refer to the values provided on the flyer of the kit.

WARNINGS AND PRECAUTIONS:

- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface.
- To preserve reagent stability, seal the vials after use with their respective caps.
- Aging studies, conducted over a 3-week period at 30°C, show that the reagents can be shipped at room temperature over a short period of time, without degradation.
- The human plasma used to prepare the calibrator and controls I and II has been tested by recorded methods and is certified free of HIV antibodies, Hbs Antigen and HCV antibodies.
- The bovine plasma used to prepare the BSA has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.
- For *in vitro* use.

CD, SD, WS : H317 : May cause an allergic skin reaction.

REAGENT PREPARATION AND STABILITY:

Bring the kit at room temperature, at least 30 min before use. Store the unused reagents at 2-8°C. Vials are closed under vacuum. Remove carefully the stopper of lyophilized reagents, in order to avoid any loss of powder when opening the vials.
When appropriately used and stored, according to the recommended protocol and cautions, the kit can be used over a 1 month period, and strip by strip, if required.

1. **COAT: Micro ELISA plate:** Open the aluminium pouch and take off the required amounts 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 plastic microplate storage bag (minigrip).
2. **SD: Sample Diluent:** Ready to use. This reagent contains Proclin.
   Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
   - **4 weeks** at 2-8°C.
3. **CAL: Factor VII Calibrator:** Reconstitute each vial with **2 mL** of **"Sample Diluent"**, shake vigorously until fully dissolved, in order to obtain a plasma containing a FVII concentration "C", in % (**already diluted 1:20**).
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial is:
   - **24 hours** at room temperature (18-25°C).
   - **72 hours** at 2-8°C.
4. **CI: FVII Control I (human plasma, high):** Reconstitute each vial with **0.5 mL** of distilled water, shake vigorously until fully dissolved.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **24 hours** at room temperature (18-25°C).
   - **72 hours** at 2-8°C.
   - **2 months** frozen at -20°C or below.
5. **CII: FVII Control II (human plasma, low):** Reconstitute each vial with **0.5 mL** of distilled water, shake vigorously until fully dissolved.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **24 hours** at room temperature (18-25°C).
   - **72 hours** at 2-8°C.
   - **2 months** frozen at -20°C or below.
6. **IC: Anti-(h)-FVII-HRP immunoconjugate:** Reconstitute each vial with **7.5 mL of Conjugate Diluent** at least 15 min before use. Let the pellet to be completely dissolved before use, and shake the vial in order to homogenize the content.
   Stability of reconstituted reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **24 hours** at room temperature (18-25°C).
   - **4 weeks** at 2-8°C.
7. **CD: Conjugate Diluent:** Ready to use. This reagent contains Proclin. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **4 weeks** at 2-8°C.
8. **WS: Wash Solution:** Incubate, if necessary, the vial in a water bath, at **37°C**, until complete dissolution of crystals. Shake the vial and dilute the amount required **1:20** in distilled water (the 50 mL contained in the vial allow to prepare 1 litter of Wash Solution).
   Stability of the wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **4 weeks** at 2-8°C.

   Stability of the dilute wash solution, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **7 days** at 2-8°C.

   This reagent contains Proclin.
9. **TMB:** Ready to use. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
   - **4 weeks** at 2-8°C.
10. **SA: Stop Solution:** Stop solution containing 0.45M sulfuric acid, ready to use. See CAUTIONS AND WARNINGS. Stability of reagent, provided that any contamination or evaporation is avoided, kept in its original vial:
    - **4 weeks** at 2-8°C.

STORAGE CONDITIONS:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

Reagents:
- Distilled water.

Materials:
- **8-channel pipettes** allowing dispensing volumes of 50-300 µL**.**
- **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.

SPECIMEN COLLECTION AND PREPARATION:

Specimens should be prepared and stored in accordance with applicable local guidelines.

- Specimens:

Human plasma obtained from anticoagulated blood (trisodium citrate). EDTA collected human plasma may also be used. The storage conditions are the same with citrated plasma.

- Collection:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M) by clean venipuncture. Discard the first tube.

- Centrifugation:

Within 2 hours, use a laboratory-validated method to obtain platelet-poor plasma, for example at least 15 minutes at 2500g at room temperature (18-25°C) and allow the plasma to settle in a plastic tube.

- Plasma storage:
  - 8 hours at room temperature (18-25°C).
  - 6 months at -20°C.

Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

PROCEDURE:

Assay procedure:

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

2. The plasma or specimen to test are analyzed **diluted at 1:20** in the Sample Diluent (SD). For expected FVII concentrations higher than "**C**" in %, dilute at 1:40 **(D=40)**, or more**.** For low or very low FVII concentrations lower dilutions can be used.

3. Using the **FVII Calibrator** with a FVII concentration "**C**" (greater than or equal to 100%, according to the lot), provided in the kit, prepare the following standard solutions:

FVII concentration (%)	C	C/2	C/4	C/10	C/20	0
Vol. of Plasma FVII calibrator	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 for homogenization.
The standard dilutions are stable for **4 hours** at room temperature (18-25°C).

4. Remove the required number of strips from the aluminium pouch and 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 controls or tested sample or sample diluent (blank)	**50 µL**	Introduce **immediately** the calibrator 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.
Mix gently on a plate shaker or manually. Incubate for 1 hour at room temperature (18-25°C). Then, wash the plate.		
Wash Solution (WS) (20 fold diluted in distilled water before use)	**300 µL**	Proceed to 5 successive washings **(b).**
TMB/H_2O_2 Substrate	**200 µL**	Immediately, introduce the substrate into the wells **(b).** ***Note:*** The substrate distribution, row by row, must be accurate **(c, d).**
Incubate for exactly 5 minutes at room temperature (18-25 °C) (d).		
0.45 M Sulfuric Acid SA (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 the obtained OD at 450 nm. Subtract the blank value (e).		

Remarks:

a) Distribute controls and tested specimen as rapidly as possible (≤10 minutes), in order to obtain an homogeneous immunological kinetics for antibodies binding. A too long delay between the distribution of the first and the last wells may induce an influence of immunological kinetics and produce wrong results.

b) Never let the wells of ELISA plates empty between the addition of the reagents or following the washing step in order to preserve insoluble proteins. The next reagent must be added within 3 minutes, in order to prevent the plate from drying, which could damage the immobilized 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.

d) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used. An incubation temperature (18-25°C) must be respected. Results are affected by a too high (>25°C) or too low (<18°C) temperature, and measured OD at 450 nm are then too high or too low. It has to be considered when analyzing the results. In the same way, if a microplate shaker is used, it should be used only at the beginning of each step (sample introduction, immunoconjugate introduction, stop solution introduction), for 1 to 2 minutes, in order to obtain a good homogeneity. OD 450 values generated in the assay are significantly increased if shaking is used throughout the incubation steps.

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

TWO STEP METHOD:

- The assay of FVII Ag can also be performed with a "two step" method. The calibration curve is from **0 to C %** (as for the one step method). The FVII calibrator (CAL) being reconstituted with **2 mL** of Sample Diluent (SD), **then diluted 1:5 in Sample Diluent (SD).** From this 1:5 diluted solution (solution at **C%**, corresponding to a plasma already diluted **1:100**), prepare calibration dilutions as for the one-step method.
- The immunoconjugate (IC) must be reconstituted with **7.5 mL** of Conjugate Diluent (CD).
- Tested plasma and control I and II must be assayed at a 100 fold (**1:100**) dilution in Sample Diluent (SD) or at higher dilutions if higher concentration of C% are expected.
- In each microwell of ELISA plate, introduce **200 µL** of the calibration solution (prepared as for the one step method) or **200 µL** of the diluted **1:100** tested plasma (or more if necessary). Following a **1 hour** incubation at room temperature (18-25°C), wash the plate and introduce **200 µL**/well of immunoconjugate (IC). Incubate 1 hour at room temperature (18-25°C), wash the plate, and introduce TMB subtrate (**200 µL/well**). Stop the colour development developed for **exactly 5 min** with **50 µL** of 0.45M sulfuric acid (SA) per well and OD at 450 nm is 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.

RESULTS:

- For the manual endpoint method, plot the calibration curve, with the **OD 405 nm** along the Y-axis and the FVII concentration, expressed as %, along the X-axis by choosing the "best fit" interpolation mode (refer to the flyer in the kit).
- Users must construct their own calibration curve, obtained using their calibrator dilutions. 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**, the concentrations are directly deduced from the calibration curve.
- Alternatively, an ELISA software (i.e., Dynex, Biolise, etc...) can be used for the calculation of concentrations.

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

LIMITATIONS:

- In order to get the optimal assay performances and adhere to specifications, the procedural instructions validated by HYPHEN BioMed must be strictly respected. It is responsibility of the user laboratory to validate any modification to those instructions for use.
- Any reagent presenting an unusual appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- Any plasma displaying a coagulum or showing signs of contamination must be rejected.

PERFORMANCE:

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

SYMBOLS:

Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

Changes compared to the previous version.