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ZYMUTEST Factor XIII-A

 # RK034A

(Complete ELISA kit for Factor XIII-A)

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

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INTENDED USE:

The ZYMUTEST Factor XIII-A kit is a one step "two site" immuno-assay for measuring human Factor XIII (subunit A) (FXIII-A) in plasma, or in any fluid where FXIII can be present. **This kit is for research use only and should not be used for patient diagnosis or treatment.**

ASSAY PRINCIPLE:

First, the immunoconjugate, which is a polyclonal antibody specific for FXIII-A coupled to horse radish peroxidase (HRP), is introduced into the microwells coated with a polyclonal antibody specific for FXIII-A. Then, the diluted tested sample is immediately introduced, and the immunological reaction starts. When present, FXIII-A 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 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 FXIII-A in the tested sample.

TEST SAMPLE:

– Trisodium Citrate anticoagulated human plasma.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with a sheep polyclonal antibody specific for human FXIII-A, 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 Calibrator**, lyophilised. When restored with **2 ml** of Sample Diluent, a plasma containing a concentration "**C"** (expressed in %) of human FXIII-A is obtained. This concentration (in the range 120-200% according to the lot), established by reference to a normal citrated human plasma pool, is accurately determined for each lot. The exact concentration is indicated on the flyer provided with each 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).

Nota : The FXIII-A concentrations and acceptancy 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)-FXIII-HRP immunoconjugate**, a polyclonal antibody coupled to 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.

***Nota*:** 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.

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 Calibrator:** restore each vial with **2 ml** of Sample Diluent in order to obtain a plasma containing a FXIII-A concentration **"C",** already diluted 1:50 (fifty fold). This solution is stable for at least **8 hours at room temperature.**
4. **Plasma Control I (High)** (human plasma, high): restore with **0.5 ml** distilled water.
5. **Plasma Control II (Low)** (human plasma, low): restore with **0.5 ml** distilled water.

Nota: when restored, controls are stable for **8 hours** at room temperature, or **24 hours at 2-8°C,** or **1 month** 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. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

6. **Anti-(h)-FXIII-HRP immunoconjugate**: each vial must be restored with **4 ml of Conjugate Diluent**. Let the pellet to be completely dissolved 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.

***Nota*:** Bring the kit at room temperature, at least 30 min. before use. Store the unused reagents at 2-8°C.
The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

PROCEDURE:

Specimen collection:

Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within 4 hours or stored frozen at –20°C or colder for up to 3 months, and thawed for 15 min. at 37°C just before use.

Refer to GEHT or NCCLS guidelines for further information on specimen collection, handling and storage.

Tested plasma or sample or controls:

The sample must be tested diluted **fifty fold (1:50)** in the Sample Diluent. For expected FXIII concentrations higher than "**C**" (in %), plasma or samples must be tested at a higher dilution, i.e., **1:100 (D=100), or more.** For low or very low FXIII concentrations lower dilutions can be used.
Plasma **Controls I and II** must be tested diluted **fifty fold (1:50)**, with Sample Diluent.

Calibration:
Using the **Plasma Calibrator,** with a FXIII-A concentration "**C**" (in the range 120-200% according to the lot used, and indicated on the lot flyer), provided in the kit, prepare the following standard solutions.

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

Mix gently for a complete homogenisation.
The standard dilutions are stable for at least **4 hours** at room temperature.

Assay procedure:
Remove the required number of 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
Conjugate anti (h)-FXIII-HRP. (Restored with 4ml of conjugate Diluent)	**100 µl**	Introduce the Anti-(h)-FXIII- HRP immunoconjugate in the micro ELISA plate wells
Plasma calibrator or tested sample or sample diluent (blank)	**100 µl**	Introduce **immediately** the standard solutions or the tested samples in the corresponding micro ELISA plate well
Mix gently on a plate shaker or manually and incubate for 1 hour at room temperature (18-25°C)		
Wash Solution (20 fold diluted in distilled water)	**300 µl**	Proceed to 5 successive washings using the washing instrument. **(a)**
TMB/H_2O_2 Substrate	**200 µl**	Immediately after the washing, introduce the substrate into the wells. ***Note:*** The substrate distribution, row by row, must be accurate and at exact time intervals **(b, c)**.
Incubate for exactly 5 minutes at room temperature (18-25 °C) (c)		
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 **(b).**
Wait for **10 minutes** in order to allow the colour to stabilize and measure absorbance at **450 nm (A450)**. **(d)**.		

Note:

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

a. 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.
b. 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.
c. Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.
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 (or on a bilogarithmic graph paper), plot the **FXIII concentrations (%)** on abscissa and the corresponding absorbances (**A450**) on ordinates.
- Users must construct their own calibration curve, obtained using their calibrator dilutions (See model on the flyer). From the curve obtained, deduce directly the FXIII concentration for the tested sample. For obtaining the FXIII concentration in a sample tested at a higher or lower dilution, this value must be multiplied by **D:50** (i.e., x**2 for D=100**, or **x 0.40 for D=20**...).
- 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.
- By definition, the 100% Factor XIII (FXIII) concentration corresponds to the concentration in a normal human citrated plasma pool, obtained by pooling plasmas from healthy males or females aged from 18 to 55 years, and out of any medication or disease.

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

BIOCHEMISTRY:

Factor XIII (FXIII) circulates in blood as a tetrameric complex composed of two types of subunits (A2B2): the A subunit (of about 83kDa) is responsible for the catalytic activity (transglutaminase), and the B subunit (of about 76kDa) may serve as a carrier protein for the A subunit. The concentration of FXIII tetramer is about 25 µg/ml in plasma.
Intracellular storage of FXIII also exists as an A2 dimer form, especially in platelets, monocytes and macrophages.
The circulating FXIII proenzyme is activated into FXIIIa by thrombin, in the presence of calcium. After separation of the two subunits, partial cleavage and conformational changes, the FXIIIA molecule is converted into a "fibrin stabilizing factor", which may also crosslink other proteins to the fibrin clot, such as fibrinogen, alpha2antiplasmin, fibronectin, vitronectin, vWF… Fibrin(ogen) has an important regulatory role in the activation of FXIII.

ASSAY CHARACTERISTICS:

- Detection threshold ≤ 10%.
- Intra-assay: 3-8%.
- Inter-assay: 5-10%.