ZYMUTEST Fibronectin

 # RK028A

(Complete ELISA kit for Fibronectin Antigen)

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

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INTENDED USE:

The ZYMUTEST Fibronectin kit is a one-step enzyme immuno-assay for measuring human Fibronectin in plasma, or in any fluid where Fibronectin can be present.
This kit is for research use only and should not be used for patient diagnosis or treatment.

ASSAY PRINCIPLE:

ZYMUTEST-Fibronectin Antigen is a one-step sandwich ELISA designed with a highly purified rabbit polyclonal antibody specific for Fibronectin.

First, the immunoconjugate, which is a polyclonal antibody specific for Fibronectin coupled to Horse Radish Peroxidase (HRP), is introduced into the microwells. Then, the diluted tested sample is immediately introduced, and the immunological reaction starts. When present, Fibronectin binds onto the polyclonal antibody coated solid phase, and fixes the antibody coupled to HRP through free epitopes. Following a washing step, the peroxidase substrate, 3,3',5,5' – Tetramethylbenzidine (TMB), 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 Fibronectin in the tested sample.

TEST SAMPLE:

- Trisodium Citrate or Na_2 EDTA anticoagulated human plasma.
- Any biological fluid where Fibronectin must be measured.

REAGENTS:

1. **COAT: Micro ELISA plate,** containing 12 strips of 8 wells, coated with a highly purified rabbit polyclonal antibody specific for Fibronectin, then stabilised; the plate is packed in an aluminium pouch hermetically sealed in presence of a desiccant.
2. **SD:** 2 vials containing 60ml of **B2F-Sample Diluent**, ready to use.
3. **Cal:** 3 vials of **Fibronectin Calibrator** lyophilised. Each vials when restored with 2.5 ml of B2F-Sample Diluent, allows obtaining a solution containing about **50 ng/ml** of human Fibronectin. The exact concentration [C] is indicated on the flyer provided in the kit
4. **CI:** 1 vial of **Fibronectin Control I** (**High),** lyophilised.
5. **CII**: 1 vial of **Fibronectin Control II** (**Low),** lyophilised.

> ***Note:*** The Fibronectin concentrations and acceptancy ranges for controls can vary from lot to lot, but are precisely indicated for each lot on the flyer provided in the kit.

6. **IC:** 3 vials of **Anti-Fibronectin-HRP immunoconjugate**, a polyclonal antibody coupled to HRP, lyophilised.
7. **CD**: 1 vial of 25 ml of **B2F-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 of kits, 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 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 microplate storage bag (minigrip).
2. **B2F-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. **Fibronectin Calibrator:** restore each vial with **2.5 ml** of B2F-Sample Diluent. This solution is stable for at least **8 hours** at room temperature.
4. **Fibronectin Control I (high)**: restore with **1 ml** B2F-Sample Diluent.
5. **Fibronectin Control II (low)**: restore with **1 ml** B2F-Sample Diluent.

> ***Note:*** when restored, controls are stable for **8 hours** at room temperature, **24 hours at 2-8°C** or **2 months** frozen **at –20°C** or below.
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> ***Warning:*** Fibronectin calibrator (3) and controls (4&5) 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-Fibronectin-HRP immunoconjugate**: each vial must be restored with **4 ml of B2F-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. **B2F-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.
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> 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 **24 hours at 2-8°C** or within **8 hours** when stored at room temperature, or stored frozen at –20°C or below for up to 6 months, and thawed for 15 min. at 37°C just before use. Thawed specimen must be tested within 8 hours.
EDTA collected human plasma may also be used. Conditions of storage are the same than those for citrated plasma.

Tested plasma or sample:

The sample must be tested diluted in order to obtain a concentration **<C ng/ml** in the B2F-Sample Diluent. For example, a human plasma must be tested at **1:8000, or 1:16000, or more** in the B2F-Sample Diluent. When high dilutions are required, proceed by successive serial dilutions (i.e., 1:10, then 1:10, etc.).
Controls I & II, restored with **1 ml** of B2F-Sample Diluent, are tested "undiluted".

Calibration:
Using the **C ng/ml Fibronectin Calibrator** provided in the kit, prepare the following standard solutions.

Fibronectin concentration (ng/ml)	C	C/2	C/5	C/10	C/20	0 ng/ml
Vol. of Fibronectin Cal at C ng/ml	1 ml	0.5 ml	0.20 ml	0.1 ml	0.05 ml	0 ml
Vol. of B2F-Sample Diluent	0 ml	0.5 ml	0.80 ml	0.9 ml	0.95 ml	1 ml

Mix gently for a complete homogenization.
The standard dilutions are stable for at least **8 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-Fibronectin-HRP. (Restored with 4 ml of B2F-Conjugate Diluent)	**100 µl**	Introduce the Anti-Fibronectin- HRP immunoconjugate in the micro ELISA plate wells
Fibronectin calibrator or controls or tested sample or B2F-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 (a)		
Wash Solution (20 fold diluted in distilled water)	**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. 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) (a)		
0.45 M Sulfuric Acid (SA)	**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)**. **(d)**.		

Note:
a) Avoid letting the plate in the bright sunlight during incubations and more particularly during colour development. A micro-ELISA plate shaker can be used.
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 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) For bichromatic readings, a reference wavelength at 690 nm or at 620 nm can be used.

RESULTS:

- On a linear graph paper plot the **Fibronectin concentration**, in **ng/ml**, on abscissae and the corresponding absorbance (**A450**) on ordinates.
- Users must construct their own calibration curve obtained using their standard dilutions. From the curve obtained (see model on the flyer), deduce the Fibronectin concentration for the tested dilution. For obtaining the Fibronectin concentration in the tested sample, this value must be **multiplied by the dilution factor**.
- For **controls I and II**, the concentrations measured are directly deduced from the 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 purposes only and not used for patient diagnosis or treatment.

BIOCHEMISTRY:

Fibronectin is a 450 kDa glycoprotein, composed of 2 disulfide-bridged chains of approximately equal size. This essential component of extracellular matrix is involved in many biological processes, and also exists in a soluble form. Plasma Fibronectin is involved in platelet aggregation, thrombus formation and wound healing, and can interact with various molecules such as fibrin (via factor XIIIa), fibrinogen, heparin, collagens, etc. The Fibronectin concentration in normal human plasma is usually about 300 ± 100 µg/ml.

STANDARDISATION:

The Fibronectin calibrator of the ZYMUTEST Fibronectin kit is established against various normal pooled citrated plasmas.

REACTIVITY:

The ZYMUTEST Fibronectin assay measures Fibronectin, and may cross-react with Fibronectin from other vertebrate species (different from rabbit), and in particular with mouse Fibronectin.

RECOMMENDATIONS:

High plasma dilutions (1:8000 or 1:16000 or more) are required when testing Fibronectin. For an improved accuracy proceed to successive serial 1:10 dilutions until the tested dilution is obtained.

REFERENCES:

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3. SA Corbett, L Lee, CL Wilson, JE Schwarzbauer, Covalent cross-linking of fibronectin to Fibrin is required for maximal cell adhesion to a fibronectin-fibrin matrix, *J Biol Chem*, 1997, **272** (40): 24999-25005.