Human Fibronectin ELISA Kit

Enzyme Immunoassay for the quantification of Human Fibronectin (FN) in milk, saliva, urine, CSF and cell culture supernatant samples

Catalog number: ARG81301

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Fibronectin (FN) is a glycoprotein present in a soluble dimeric form in plasma, and in a dimeric or multimeric form at the cell surface and in extracellular matrix. Fibronectin is involved in cell adhesion and migration processes including embryogenesis, wound healing, blood coagulation, host defense, and metastasis. The gene has three regions subject to alternative splicing, with the potential to produce 20 different transcript variants. However, the full-length nature of some variants has not been determined. [provided by RefSeq, 2008] Fibronectins bind cell surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin. Fibronectins are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Involved in osteoblast compaction through the fibronectin fibrillogenesis cell-mediated matrix assembly process, essential for osteoblast mineralization. Participates in the regulation of type I collagen deposition by osteoblasts.

Anastellin binds fibronectin and induces fibril formation. This fibronectin polymer, named superfibronectin, exhibits enhanced adhesive properties. Both anastellin and superfibronectin inhibit tumor growth, angiogenesis and metastasis. Anastellin activates p38 MAPK and inhibits lysophospholipid signaling. [UniProt]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification of Human Fibronectin in milk, saliva, urine, CSF and cell culture supernatant samples.

This assay employs the sandwich enzyme immunoassay technique. A polyclonal antibody specific for human Fibronectin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any

Fibronectin present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-conjugated antibody specific for Fibronectin is added to each well and incubate. Following a washing to remove unbound substances, streptavidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Fibronectin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Fibronectin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the kit at 2-8 °C. Antibodies and reconstituted standard should be stored at -20°C. Use the kit before expiration date.

Component	Quantity	Storage information
Antibody-coated microplate	12 x 8 wells	4°C
Human FN Standard (1.8 µg)	1 vial (Lyophilized)	4°C (store at -20°C after reconstitution)
Biotinylated Human Fibronectin antibody (50X)	120 µl	-20°C
Streptavidin-HRP (100X)	80 µl	-20°C
EIA Diluent (10X)	30 ml	4°C
20X wash buffer concentrate	2 X 30 ml	4°C
Substrate Solution (TMB)	8 ml (Ready to use)	4°C
STOP solution	12 ml (Ready to use)	4°C
Plate sealer	3 pieces	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the components at recommended temperature.
- If crystals are observed in the 20X Wash buffer or 10X EIA Diluent, warm to RT or 37°C, mix well until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.
- 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.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines.

Cell Culture Supernatants: Collect cell culture media and centrifuge at 1500 x g for 10 minutes at 4°C to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -80°C for up to 3 months. Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples 1:1 with 1X EIA Diluent (2X dilution, dilution factor =2) and assay immediately (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.

Saliva: Collect saliva using sample tube. Centrifuge samples at 800 x g for 10 minutes and assay. It is suggested dilute samples 1:19 with 1X EIA Diluent (20X dilution, dilution factor =20) and assay immediately (A 20 fold sample dilution is suggested into 1X EIA Diluent or within the range of 20x – 80x; however, user should determine optimal dilution factor depending on application needs.). Store undiluted samples at-20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Milk: Collect milk using sample tube. Centrifuge samples at 800 x g for 10 minutes. It is suggested dilute samples 1:199 (200X dilution, dilution factor =200) with 1X EIA Diluent and assay immediately (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.

CSF: Collect CSF using sample tube. Centrifuge samples at 3000 x g for 10 minutes. It is suggested dilute samples 1: 99 (100X dilution, dilution factor =100) with 1X EIA Diluent and assay immediately (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.

Note:

1. Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.
2. Refer to Dilution Guidelines for further instruction. (for reference only; please follow the insert for specific dilution suggested)
    a) 100X dilution: 4 µl sample + 396 µl 1X EIA Diluent
    b) 1,000X dilution: 40 µl 100X diluted sample from a) + 360 µl 1X EIA Diluent
    c) 10,000X dilution: 4 µl 100X diluted sample from a) + 396 µl 1X EIA Diluent
    d) 100,000X dilution: 40 µl 10,000X diluted sample from c) + 360 µl 1X EIA Diluent

REAGENT PREPARATION

- 1X EIA Diluent: Dilute 10X EIA Diluent into distilled water to yield 1X EIA Diluent buffer. (E.g. 30 ml of 10X EIA Diluent + 270 ml of 1X distilled water) If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use. Store for up to 30 days at 2-8°C.
- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 30 ml of 20X Wash buffer + 570 ml of 1X distilled water) If crystals appear in buffer, warm the buffer in warm water bath for 30 minutes or mix gently until crystals disappear. Mix well before use. The diluted wash buffer should be store at 2-8°C.
- Biotinylated Human Fibronectin (FN) antibody: Briefly spin down tube. Dilute the 50X Biotinylated Human Fibronectin antibody with 1X EIA Diluent buffer. (E.g. 20 µl of 50X Biotinylated Human Fibronectin antibody + 980 µl of 1X EIA Diluent buffer) Any remaining solution should be frozen at-20°C.
- Streptavidin-HRP conjugate: Briefly spin down tube. Dilute the 100X Streptavidin-HRP Conjugate with 1X EIA Diluent. (E.g. 20 µl of 100X Streptavidin-HRP Conjugate + 1980 µl of 1X EIA Diluent buffer) Any remaining solution should be frozen at-20°C.
- Standard peptide: Reconstitute a vial of Standard (1.8 µg) with 1.8 ml of 1X EIA buffer. Vortex. The concentration of this stock solution is 1000 ng/ml. Allow the vial to sit for 10 minutes at RT. Mix well and spin down before use. Dilute standard solutions according to the table below and

make serial dilutions of 1000 ng/ml, 250 ng/ml, 62.5 ng/ml, 15.63 ng/ml, and 3.9 ng/ml. And the 1X EIA Diluent serves as the zero standard.

Standard No.	Standard	1X EIA Buffer	Concentrations (ng/ml)
S1	1800 µl	0 µl	1000
S2	1 part of S1	3 parts	250
S3	1 part of S2	3 parts	62.5
S4	1 part of S3	3 parts	15.63
S5	1 part of S4	3 parts	3.9
B	0	1X EIA Buffer	0

ASSAY PROCEDURE

1. Prepare all reagents, standard solutions, and samples as instructed. **Bring all reagents to room temperature before use.** The assay is performed at room temperature (RT, 20-25°C). 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.
2. Add 50 µl of Human FN Standard or sample per well, gently tap the plate to mix well. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours at RT. Start the timer after the last addition.
3. Remove sealer from plate.
4. Aspirate each well and wash, repeating the process 4 times for a **total 5 washes**. Wash by filling each well with **1× Wash Buffer (300 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of

liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

5. Add **50 µl** of diluted **Biotinylated Human FN antibody** into each well, gently tap the plate to mix well. Break any bubbles that may have formed
6. Reseal the plate with sealer. Incubate for **1 hour at RT**.
7. **Wash** as according to step 4.
8. Add **50 µl** of diluted **Streptavidin-HRP solution** into each well, gently tap the plate to mix well. Break any bubbles that may have formed.
9. Reseal the plate with sealer. Incubate for **30 minutes at RT**. (Turn on the microplate reader and set up the program in advance.)
10. **Wash** as according to step 4.
11. Add **50 µl** of **TMB substrate solution** into each well l, gently tap the plate to mix well. Break any bubbles that may have formed.
12. Incubate for **12 minutes or until the optimal blue color density develops**. (Protect from light)
13. Add **50 µl** of **STOP solution** into all wells to stop the reaction. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
14. Read the OD with a microplate reader at **450 nm immediately.** (optional: read at 570 nm as reference wave length) It is recommended read the absorbance within 10 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

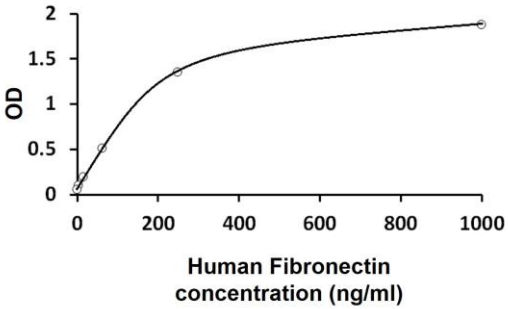
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The minimum detectable dose of human fibronectin as calculated by 2SD from the mean of a zero standard (B) was established to be 2 ng/ml.

Range

Standard Range: 3.9 - 1000 ng/ml

Recovery

88-111%

Linearity

Milk samples were serially diluted to test for linearity and the Linearity was 93-107% from 100X-400X dilution.

Intra-assay and Inter-assay precision

The CV value of intra-assay precision was 3.3 % and inter-assay precision was 9.3 %.

Cross Reactivity

The kit cross-reacts:

FN (Monkey) < 25%

This kit detects no cross-reactivity with the following factor:

FN from Bovine, Canine, Mouse, Rabbit, Rat and Swine