AssayMax Rat Fibronectin ELISA Kit

Catalog # ERF1045-1

Introduction

Fibronectin (FN) is a major component of the extracellular matrix and blood plasma, and is a specific ligand for several integrin adhesion receptors (1). FN plays an important role not only in cell adhesion (2) and wound healing (3), but also in embryogenesis (4) and hematopoiesis (5). FN is over-expressed in cardiovascular disease states such as atherosclerosis (6) and myocardial infarction (7). Reduced levels of FN have been reported in patients with Disseminated Intravascular Coagulation (DIC) and low concentrations appear to correlate with a poor prognosis (8).

Principal of the Assay

The AssayMax Rat Fibronectin ELISA kit is designed for detection of Rat Fibronectin in plasma, serum and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures fibronectin in 3.5 hours. A polyclonal antibody specific for fibronectin has been pre-coated onto a microplate. Fibronectin in standards and samples is sandwiched by the immobilized antibody and biotinylated polyclonal antibody specific for fibronectin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution.

Reagents

- **Fibronectin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against rat fibronectin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Fibronectin Standard:** Recombinant rat fibronectin in a buffered protein base (16 µg, lyophilized).
- **Biotinylated Fibronectin Antibody (100x):** A 100-fold biotinylated polyclonal antibody against fibronectin (80 µl).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (90 µl).

- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2 - 8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2 - 8^{0}C. Store reconstituted standard at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:500 into EIA Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:500 into EIA Diluent. The undiluted samples can be stored at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20^{0}C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Fibronectin Standard:** Reconstitute the 16 µg of rat fibronectin Standard with 4 ml of EIA Diluent to generate a stock solution of 4 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the Standard solution (4 µg/ml) twofold with equal volume of EIA Diluent to produce 2, 1, 0.5, 0.25, 0.125, 0.0625 and 0.03125 µg/ml. EIA Diluent serves as the zero standard (0 µg/ml).

Standard Point	Dilution	[Fibronectin] (μg/ml)
P1	1 part Standard (4 μg/ml) + 1 part EIA Diluent	2.000
P2	1 part P1 + 1 part EIA Diluent	1.000
P3	1 part P2 + 1 part EIA Diluent	0.500
P4	1 part P3 + 1 part EIA Diluent	0.250
P5	1 part P4 + 1 part EIA Diluent	0.125
P6	1 part P5 + 1 part EIA Diluent	0.063
P7	1 part P6 + 1 part EIA Diluent	0.032
P8	EIA Diluent	0.000

- **Biotinylated Fibronectin Antibody (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the antibody 1:100 with EIA Diluent.
- **EIA Diluent Concentrate (10x)**: Dilute the EIA Diluent Concentrate 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30^{0}C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 μl of Standard or sample per well. Cover wells and incubate for 2 hours. Start the timer after the last sample addition.
- Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete remove liquid at each step.
- Add 50 μl of Biotinylated Fibronectin Antibody to each well and incubate for 60 minutes.
- Wash five times with 200 μl of Wash Buffer.
- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with 200 μl of Wash Buffer.
- Add 50 μl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

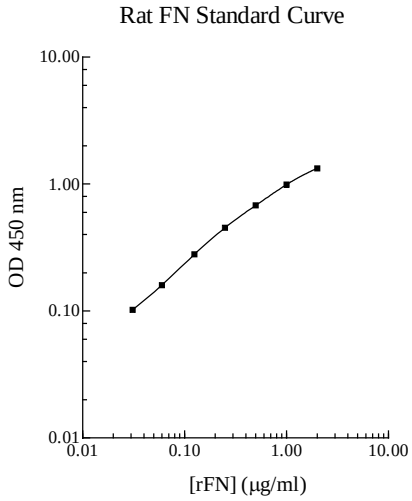
Data Analysis

- Calculate the mean value of the triplicate readings for each standard and sample.

- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or 4-parameter curve fit.
- Determine the unknown sample concentration from the Standard Curve. Multiply the plasma or serum value by the dilution factor of 500.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of fibronectin is typically 20 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1 % and 6.8% respectively.
- This kit can be used for rat samples.

References

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