# Rat Fibronectin ELISA Kit

Catalog Number:
HFB21-K01 (1 x 96 wells)
*For Research Use Only. Not for use in diagnostic procedures.*
*v. 1.0*

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## INTENDED USE

The Eagle Biosciences Rat Fibronectin ELISA Kit is intended for the quantitation of Rat FN1 concentrations in cell culture supernates, serum and plasma (heparin, EDTA, citrate). The Eagle Biosciences Rat Fibronectin ELISA Kit is for research use only and not for diagnostic or therapeutic procedures.

## INTRODUCTION

Fibronectin (FN) also known as LETS, is identified on the surfFN of fibroblasts by labeling with radioactive compounds or specific antibodies. Fibronectin is a 430,000-dalton dimeric glycoprotein that exists in 2 forms, termed cellular and plasma fibronectin. Cellular and plasma fibronectins are heterodimers consisting of similar but not identical polypeptides. These two forms of FN differ in biologic activity. Fibronectins bind cell surfFNs and various compounds including collagen, fibrin, heparin, DNA, and actin. Because fibronectin stimulates endocytosis in several systems and promotes the clearance of particulate material from the circulation, it could function in the clearance of C1q-coated material such as immune complexes or cellular debris. Fibronectins are involved in cell adhesion, cell motility, opsonization, would healing, and maintenance of cell shape. LETS, encoded on chromosome 8, is responsible for the LETS protein expression in humans. Because LETS has been implicated in tumorigenicity and cellular transformation, it is of interest that rearrangement or modifications in the number of chromosome 8 have been associated with certain forms of cancer. The standard used in this kit is isolated from rat plasma with the molecular mass of 200-250KDa.

## PRINCIPLE OF THE ASSAY

The Rat Fibronectin ELISA Kit is a solid phase immunoassay specially designed to measure Rat FN1 with a 96-well strip plate that is pre-coated with antibody specific for FN1. The detection antibody is a biotinylated antibody specific for FN1. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Rat FN1 with immunogen: Expression system for standard: from plasma. The kit is analytically validated with ready to use reagents.

To measure Rat FN1, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly proportional to Rat FN1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Rat FN1 in the sample.

| | |
|---|---|
| **Reactive Species** | Rat |
| **Size** | 96 wells/kit, with removable strips |
| **Description** | Sandwich High Sensitivity ELISA kit for Quantitative Detection of Rat Fibronectin. 96wells/kit, with removable strips. |
| **Sensitivity** | <15pg/ml<br>*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration. |
| **Detection Range** | 156pg/ml-10000pg/m |

**Storage Instructions** Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

**TECHNICAL DETAILS**

| | |
|---|---|
| **Capture/Detection Antibodies** | The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. |
| **Specificity** | Natural and recombinant Rat FN1 |
| **Immunogen** | Expression system for standard: from plasma |
| **Cross Reactivity** | There is no detectable cross-reactivity with other relevant proteins. |

**NOTICE BEFORE APPLICATION**

Please read the following instructions before starting the experiment.

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

**KIT COMPONENTS/MATERIALS PROVIDED**

| Description | Quantity | Volume |
|---|---|---|
| Anti-Rat FN1 Precoated 96-well strip microplate | 1 | 12 strips of 8 wells |
| Rat FN1 Standard | 2 | 10ng/tube |
| Rat FN1 Biotinylated antibody (100x) | 1 | 130 µl |
| Avidin-Biotin-Peroxidase Complex (100x) | 1 | 130 µl |
| Sample Diluent | 1 | 30ml |
| Antibody Diluent | 1 | 12ml |
| Avidin-Biotin-Peroxidase Diluent | 1 | 12ml |
| Color Developing Reagent (TMB) | 1 | 10ml |
| Stop Solution | 1 | 10ml |
| Wash Buffer Powder | 1 | Pack |
| Plate Sealers | 4 | Piece |

**REQUIRED MATERIALS THAT ARE NOT SUPPLIED**

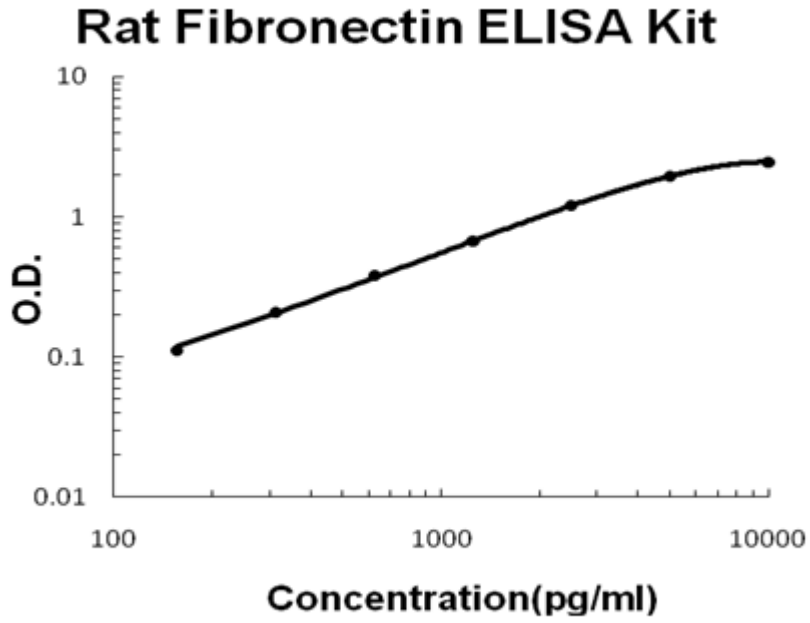
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

**STANDARD CURVE EXAMPLE**

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

| Concentration (pg/ml) | 0 | 156 | 313 | 625 | 125000 | 25000 | 5000 | 10000 |
|---|---|---|---|---|---|---|---|---|
| O.D. | 0.012 | 0.111 | 0.208 | 0.381 | 0.668 | 1.201 | 1.952 | 2.461 |

**Rat Fibronectin ELISA Kit**




A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

**INTRA/INTER ASSAY VARIABILITY**

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.
Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

Intra-Assay Precision

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| N | 16 | 16 | 16 |
| Mean(pg/ml) | 342 | 1852 | 4326 |
| Standard Deviation | 23.25 | 85.49 | 190.34 |
| CV (%) | 6.8% | 4.6% | 4.4% |

Inter-Assay Precision

| Sample | 1 | 2 | 3 |
|---|---|---|---|
| N | 24 | 24 | 24 |
| Mean(pg/ml) | 361 | 1834 | 4010 |
| Standard Deviation | 29.96 | 102.7 | 216.54 |
| CV (%) | 8.3% | 5.6% | 5.4% |

**REPRODUCIBILITY**

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

| Lots | Lot1 (pg/ml) | Lot2 (pg/ml) | Lot3 (pg/ml) | Lot4 (pg/ml) | Mean (pg/ml) | Standard Deviation | CV (%) |
|---|---|---|---|---|---|---|---|
| Sample 1 | 342 | 322 | 342 | 345 | 337 | 9.17 | 2.7% |
| Sample 2 | 1852 | 1882 | 1790 | 2001 | 1878 | 79.72 | 4.2% |
| Sample 3 | 4326 | 4126 | 3772 | 4098 | 4080 | 198.63 | 4.8% |

*number of samples for each test n=16.

**PREPARATION BEFORE THE EXPIREMENT**

| Item | Preparation |
|---|---|
| All reagents | Bring all reagents to 37°C prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C. |
| Wash Buffer | Disolve the wash buffer powder in 1000ml of water to make 1X PBS wash buffer. |
| Biotinylated Anti-Rat FN1 antibody | It is recommended to prepare this reagent immediately prior to use by diluting the Rat FN1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| Avidin-Biotin-Peroxidase Complex | It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation. |
| Rat FN1 Standard | It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10ng of lyophilized Rat FN1 standard for each experiment. Gently spin the vial prior to use. |

| | |
|---|---|
| | Reconstitute the standard to a stock concentration of 10ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions. |
| Microplate | The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging. |

**DILUTION OF RAT FN1 STANDARD**

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –10000pg/ml, #2 –5000pg/ml, #3 – 2500pg/ml, #4 – 1250pg/ml, #5 – 625pg/ml, #6 –312.5pg/ml, #7 – 156.25pg/ml, #8 – 0.0 (Blank).
2. For standard #1, add 1000µl of undiluted standard stock solution to tube #1.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

**SAMPLE PREPARATION AND STORAGE**

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

| Sample Type | Procedure |
|---|---|
| Cell culture supernatants | Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C. |
| Serum | Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C. |
| Plasma | Collect plasma using heparin, EDTA or citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C.<br>*Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site. |

**SAMPLE DILUTION**

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µl of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

**ASSAY PROTOCOL**

It is recommended that all reagents and materials be equilibrated to 37°C/room temperature prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Rat FN1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.

## DATA ANALYSIS

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logisticcurve.assay.

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

### Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.