

**Rat Fibronectin ELISA Kit
(rFN-ELISA)****Cat. No. EK0350***96 Tests in 8 x 12 divisible strips***Background**

Fibronectin (FN), also known as LETS, is identified on the surface of fibroblasts by labeling with radioactive compounds or specific antibodies. FN is a 430,000-dalton dimeric glycoprotein that exists in two forms, called 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 surfaces and various compounds including collagen, fibrin, heparin, DNA, and actin and are involved in cell adhesion, cell motility, opsonization, wound healing, and maintenance of cell shape. Because fibronectin stimulates endocytosis in several systems and promotes the clearance of particulate material from the circulation, it may function in the clearance of C1q-coated material such as immune complexes or cellular debris. FN, encoded on chromosome 8, has been implicated in tumorigenicity and cellular transformation, findings which are corroborated by fact that the 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 human plasma with the molecular mass of 200-250KDa.

ScienCell's rat Fibronectin ELISA Kit is based on standard sandwich enzyme-linked immunosorbent assay technology. Human Fibronectin-specific polyclonal antibodies are pre-coated onto 8 x 12 divisible strips. The human specific detection polyclonal antibodies are biotinylated. The test samples and biotinylated detection antibodies are subsequently added to the wells and then washed with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. HRP substrate TMB is used to visualize HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The intensity of yellow is proportional to the amount of rat Fibronectin captured in strips.

Size	96 Tests in 8 x 12 divisible strips
Assay type	Sandwich ELISA
Range	156 pg/ml- 10,000 pg/ml
Sensitivity	< 15 pg/ml
Specificity	No detectable cross-reactivity with any other cytokine.
Storage	Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles
Shipping	Shipped on gel ice.

Expiration	Four months at 4°C and eight months at -20°C.
Application	For quantitative detection of rat Fibronectin in serum, plasma, body fluids, tissue lysates or cell culture supernatants.
Kit components	1. Lyophilized rat Fibronectin standard: 10 ng/tube×2. 2. 8 x 12 divisible strips pre-coated with anti- rat Fibronectin antibody. 3. Sample diluent buffer: 30 ml 4. Biotinylated anti- rat Fibronectin antibody: 130µl, dilution 1:100. 5. Antibody diluent buffer: 12ml. 6. Avidin-Biotin-Peroxidase Complex (ABC): 130µl, dilution 1:100. 7. ABC diluent buffer: 12ml. 8. TMB color developing agent: 10ml. 9. TMB stop solution: 10ml.
Materials Required But Not Provided	1. Microplate reader. 2. Automated plate washer. 3. Adjustable pipettes and pipette tips. Multi-channel pipettes are recommended for large number of samples. 4. Clean tubes and Eppendorf tubes. 5. Washing buffer (neutral PBS or TBS). Preparation of 0.01M TBS: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L. Preparation of 0.01 M PBS: Add 8.5g sodium chloride, 1.4g Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.
Usage	This product is for research use only. It is not approved for use in humans, animals, or <i>in vitro</i> diagnostic procedures.

Reference

1. Kornblihtt, A. R.; Umezawa, K.; Vibe-Pedersen, K.; Baralle, F. E. Primary structure of human fibronectin: differential splicing may generate at least 10 polypeptides from a single gene. EMBO J. 4: 1755-1759, 1985.
2. Bing, D. H.; Almeda, S.; Isliker, H.; Lahav, J.; Hynes, R. O. Fibronectin binds to the C1q component of complement. Proc. Nat. Acad. Sci. 79: 4198-4201, 1982.
3. Owerbach, D.; Doyle, D.; Shows, T. B. Genetics of the large, external, transformation-sensitive (LETS) protein: assignment of a gene coding for expression of LETS to human chromosome 8. Proc. Nat. Acad. Sci. 75: 5640-5644, 1978.

Protocol for Rat Fibronectin ELISA (96 well format)

Notes before you begin

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color developing agent should be colorless and transparent before using.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. A duplicate well assay is recommended for both standard and samples.

5. Do not let wells dry, as this will inactivate active components on strips.
6. Do not reuse tips and tubes to avoid cross contamination.
7. Avoid using reagents from different batches.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before use.

Preparation

Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C.

Avoid repeated freeze-thaw cycles.

- **Cell culture supernatants, tissue lysates or body fluids:** Remove particulates by centrifugation, assay immediately or aliquot and store at -20°C.
- **Serum:** Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.
- **Plasma:** Collect plasma using EDTA, citrate as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Analyze immediately or aliquot and store frozen at -20°C. Heparin is not recommended as the anticoagulant.

Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. **The sample must be well mixed with the diluent buffer.**

- **High target protein concentration (100-1000 ng/ml).** The working dilution is 1:100. i.e. Add 1 µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (10-100 ng/ml).** The working dilution is 1:10. i.e. Add 10 µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (156-10000 pg/ml).** The working dilution is 1:2. i.e. Add 50 µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤ 156 pg/ml).** No dilution necessary, or the working dilution is 1:2.

Reagent Preparation and Storage

A. Reconstitution of the rat Fibronectin standard: Fibronectin standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of Fibronectin standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

- 10000 pg/ml of rat Fibronectin standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 minutes and mix thoroughly.
- 5000pg/ml → 156pg/ml of rat Fibronectin standard solutions: Label 6 Eppendorf tubes with 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml, respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 10,000pg/ml Fibronectin standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-rat Fibronectin antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Biotinylated anti-rat Fibronectin antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly.

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly.

Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard Fibronectin detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of Fibronectin amount in samples.

1. Aliquot 0.1ml per well of the 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 313pg/ml, 156pg/ml rat Fibronectin standard solutions into the pre-coated strips. Add 0.1ml of the sample diluent buffer into the control well (**blank well**). Add 0.1ml of each properly diluted sample of rat serum, plasma, body fluids, tissue lysates or cell culture supernatants to each empty well. See “**Sample Dilution Guideline**” above for details. We recommend that each rat Fibronectin standard solution and each sample is measured in duplicate.
2. Seal the strips with the cover and incubate at 37°C for 90 minutes.
3. Remove the cover, discard strips’ contents, and blot the strips onto paper towels or other absorbent material. **Do NOT** let the wells completely dry at any time.
4. Add 0.1ml of biotinylated anti-rat Fibronectin antibody working solution into each well and incubate the strips at 37°C for 60 minutes.
5. Wash strips 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 minute. Discard the washing buffer and blot the strips onto paper towels or other absorbent material. (**Strips Washing Method:** Discard the solution in the strips without touching the side walls. Blot the strips onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the strips onto paper towels or other absorbent material).
6. Add 0.1ml of prepared ABC working solution into each well and incubate the strips at 37°C for 30 minutes.
7. Wash strips 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 minutes. Discard the washing buffer and blot the strips onto paper towels or other absorbent material.(See Step 5 for strips washing method).
8. Add 90 µl of prepared TMB color developing agent into each well and incubate strips at 37°C in dark for 25-30 minutes (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated rat Fibronectin standard solutions; the other wells show no obvious color).
9. Add 0.1ml of prepared TMB stop solution into each well. The color changes into yellow immediately.
10. Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The rat Fibronectin concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the strips at 37°C for 90 minutes. Do not wash.
2. Add biotinylated antibodies and incubate the strips at 37°C for 60 minutes. Wash strips 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the strips at 37°C for 30 minutes. Wash strips 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the strips at 37°C in dark for 25-30 minutes.
5. Add TMB stop solution and read.

Typical Data Obtained from Rat Fibronectin

(TMB reaction incubate at 37°C for 25 minutes)

Concentration (pg/ml)	0.0	156	313	625	1250	2500	5000	10000
Absorbance (450 nm)	0.060	0.129	0.154	0.207	0.328	0.665	1.120	1.971

Typical Rat Fibronectin ELISA Kit Standard Curve

This standard curve was generated for demonstration purpose only. A standard curve must be run with each assay.

