FN1 ELISA Kit (Human)
(OKBB00145)
Lot# KH0066

Instructions for Use

For the quantitative measurement of Human FN1 in cell culture supernatants, serum and plasma (heparin, EDTA, citrate).

Lot to lot variations can occur. Refer to the manual provided with the kit.

This product is intended for research use only.

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1. Background

Principle

Aviva Systems Biology FN1 ELISA Kit (Human) (OKBB00145) is based on standard sandwich enzyme-linked immune-sorbent assay technology. A rabbit polyclonal antibody specific for FN1 has been pre-coated onto 96-wellplate (12 x 8 Well Strips). Standards (from plasma) and test samples are added to the wells, incubated and removed. A biotinylated detector antibody specific for FN1 is added, incubated and followed by washing. Avidin-Biotin-Peroxidase Complex is then added, incubated and unbound complex is washed away. An enzymatic reaction is visualized through the addition of TMB substrate which is catalyzed by HRP to produce a blue color product that changes to yellow after adding acidic stop solution. The density of yellow coloration read by absorbance at 450 nm and is quantitatively proportional to the amount of sample Human FN1 captured in the well.

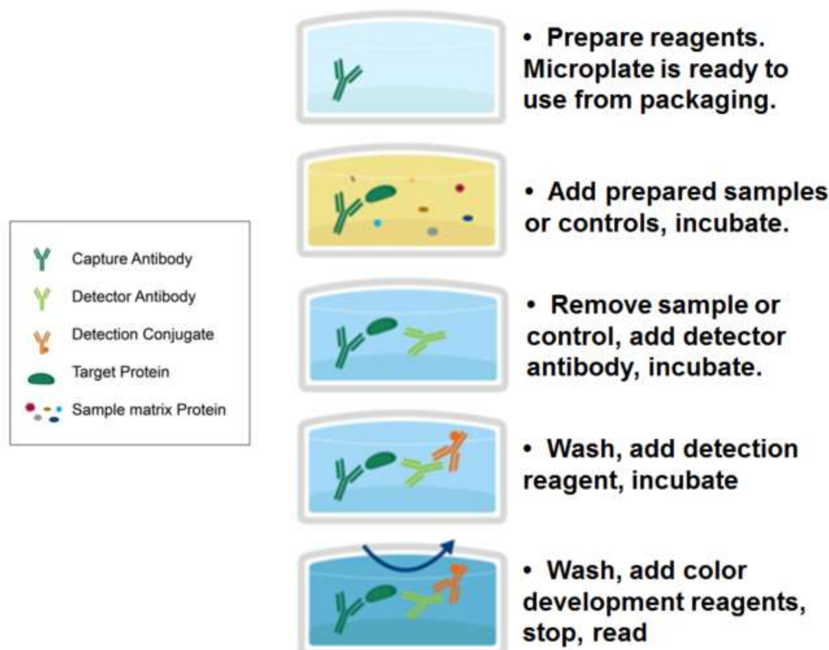
Background

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 human plasma with the molecular mass of 200-250KDa.

General Specifications

General Specifications	
Range	0.156 – 10 ng/mL
Sensitivity	< 0.01 ng/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Natural and recombinant Human FN1 UniProt ID: P02751 GeneID: 2335 Target Alias: CIG, Cold-insoluble globulin, DKFZp686F10164, DKFZp686H0342, DKFZp686I1370, DKFZp686O13149, ED-B, Fibronectin, FINC, FN, FNZ, GFND, GFND2, LETS, MSF
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

- Upon receipt store kit at -20°C until the expiration date. Avoid multiple freeze/thaw cycles.

4. Kit Components

- The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
96-Well plate Pre-coated with Anti-Human FN1 Antibody	96 Wells (12 x 8 Well Strips)	Store at -20°C until expiration date
Lyophilized Recombinant Human FN1 standard	2 x 10 ng	
100X Biotinylated Anti-Human FN1 Antibody	1 x 100 µL	
100X Avidin-Biotin-Peroxidase Complex (ABC)	1 x 100 µL	
Sample Diluent Buffer	1 x 30 mL	
Antibody Diluent Buffer	1 x 12 mL	
ABC Diluent Buffer	1 x 12 mL	
TMB Color Developing Agent	1 x 10 mL	
TMB Stop Solution	1 x 10 mL	
10X Wash Buffer	1 x 30 mL	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Inspect all reagents prior to use. Components should contain no particulates or cloudiness and should be colorless.
- Prior to using the kit, briefly spin component tubes to collect all reagent at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results in inter- intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception) and perform all incubations at 37°C.
- Pipetting less than 1 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing precipitates or fibrin strands or which are hemolytic of lipemic might cause inaccurate results due to interfering factors.

8. Reagent Preparation

• Equilibrate all materials to room temperature prior to use and use immediately.

8.1 Human FN1 Assay Standards

8.1.1 Prepare the FN1 standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2 Reconstitute one vial of the provided 10 ng **Lyophilized FN1 Standard** for each experiment. Prepare the stock 10 ng/mL Standard by reconstituting one tube of **Lyophilized FN1 Standard** as follows:

8.1.2.1 Gently spin or tap the vial at 6,000 – 10,000 rpm for 30 seconds to collect all material at the bottom.

8.1.2.2 Add 1 mL of **Sample Diluent** to the vial.

8.1.2.3 Seal the vial then mix gently and thoroughly.

8.1.2.4 Leave the vial at ambient temperature for 15 minutes.

8.1.3 Prepare a set of seven serially diluted standards as follows:

8.1.3.1 Label tubes with numbers 2 – 8.

8.1.3.2 Use the undiluted 10 ng/mL **FN1 Standard** as the high standard point (Tube #1).

8.1.3.3 Add 300 µL of **Sample Diluent** to Tube #'s 2 – 8.

8.1.3.4 Prepare **Standard #2** by adding 300 µL of 10 ng/mL **FN1** (Tube #1) to Tube #2. Mix gently and thoroughly.

8.1.3.5 Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6 Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7 Tube #8 is a blank standard (only **Sample Diluent**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	10 ng Lyophilized FN1 Standard	NA	1,000	1,000	10 ng/mL
2	10 ng/mL	300	300	600	5.0 ng/mL
3	5.0 ng/mL	300	300	600	2.5 ng/mL
4	2.5 ng/mL	300	300	600	1.25 ng/mL
5	1.25 ng/mL	300	300	600	0.625 ng/mL
6	0.625 ng/mL	300	300	600	0.313 ng/mL
7	0.313 ng/mL	300	300	600	0.156 ng/mL
8	NA	0	300	300	0.0 (Blank)

8.2 1X Biotinylated Anti-Human FN1 Antibody

8.2.1 Prepare the **1X Biotinylated Anti-Human FN1 Antibody immediately** prior to use by diluting the **100X Biotinylated Anti-Human FN1 Antibody** 1:100 with Antibody Diluent Buffer.

8.2.2 For each well to be used in the experiment prepare 100 µL by adding 1 µL of **100X Biotinylated Anti-Human FN1 Antibody to** 99 µL Antibody Diluent Buffer.

8.2.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.3 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.3.1 Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC) 1:100** with **ABC Dilution Buffer**.

8.3.2 For each well to be used in the experiment prepare 100 µL, by adding 1 µL of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 99 µL ABC Dilution Buffer.

8.3.3 Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure.

8.4 1X Wash Buffer

8.4.1 Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.4.2 Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.

8.4.3 Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4 Store the **1X Wash Buffer at** room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5 Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation

9.1 Sample Preparation and Storage

- Store samples to be assayed at 2-8°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.
- Clear samples by centrifugation as follows:

  - **Cell culture supernatants** - Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
  - **Serum** - Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
  - **Plasma** - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

9.2 Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range.

- Prepare a 150 µL sample for each replicate to be assayed.
- Dilute samples with **Sample Diluent Buffer**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.
- Refer to the following table for recommended sample dilution guidelines based on the dynamic range of this kit:

Estimated Sample Target Concentration		Dilution Level	Sample Volume For Two Replicates	Sample Diluent Buffer For Two Replicates
High Concentration	100-1,000 ng/mL	1:100	1 µL	99 µL
Medium Concentration	10-100 ng/mL	1:10	10 µL	90 µL
Low Concentration	156-10,000 pg/mL	1:2	50 µL	50 µL
Very Low Concentration	≤ 156 pg/mL	1:2 or No Dilution	-	-

10. Assay Procedure

• The ABC Working Solution, TMB Color Developing Agent and TMB Stop Solution must be kept warm at 37°C for 30 minutes prior to use. Equilibrate all other reagents and materials to ambient room temperature prior to use in the procedure.

• To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

10.1 Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.

10.2 Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.

10.3 Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.4 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5 Add 100 µL of prepared **1X Biotinylated Anti-Human FN1 Antibody to each well.**

10.6 Cover with the well-plate lid and incubate at 37°C for 60 minutes.

10.7 Wash plate 3 times with **1X Wash Buffer as** follows:

- 10.7.1 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
- 10.7.2 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.7.3 Add 300 µL of **1X Wash Buffer** to each assay well.
- 10.7.4 Incubate for 1 minute.
- 10.7.5 Repeat steps 10.7.1 through 10.7.4 **two** more times.

10.8 Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC) into** each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.9 Wash plate 5 times with **1X Wash Buffer as** follows:

- 10.9.1 Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.
- 10.9.2 Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.
- 10.9.3 Add 300 µL of 1X Wash Buffer to each assay well.
- 10.9.4 Incubate for 1 minute.
- 10.9.5 Repeat steps 10.9.1 through 10.9.4 **four** more times.

10.10 Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate at 37°C in the dark for 15-25 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.11 Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.

10.12 Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

$$(\text{Relative } OD_{450}) = (\text{Well } OD_{450}) - (\text{Mean Blank Well } OD_{450})$$

The standard curve is generated by plotting the mean replicate Relative **OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The Human FN1 concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software.

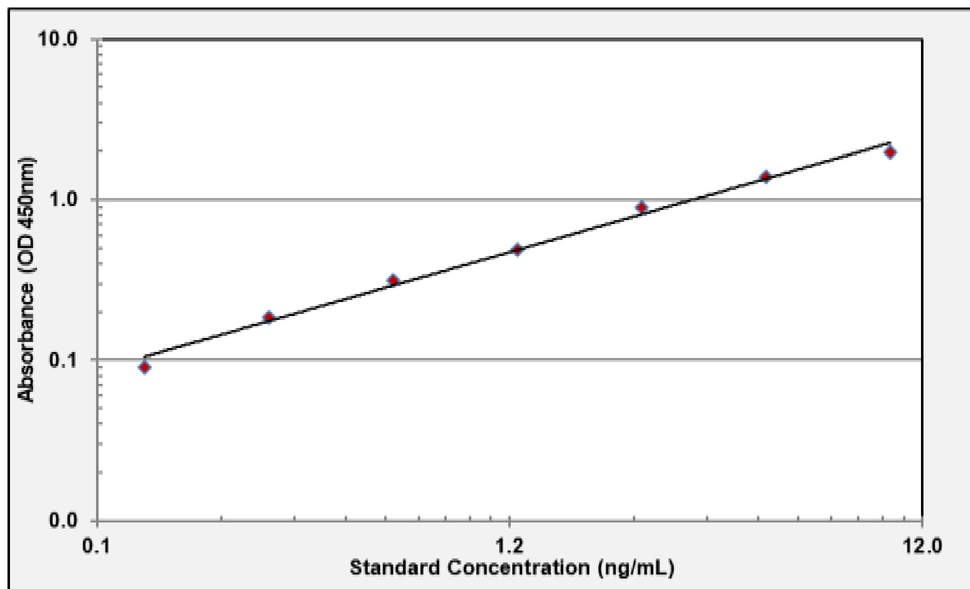
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1 Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
standard Concentration (ng/mL)	0	0.156	0.313	0.625	1.25	2.5	5.0	10
OD_{450}	0.052	0.091	0.186	0.317	0.490	0.890	1.385	1.990

12.2 Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3 Reproducibility

	Intra-Assay			Inter-Assay		
Sample ID	1	2	3	1	2	3
n =	16	16	16	24	24	24
Mean Measured Concentration (pg/mL)	312	1118	5412	341	1105	4997
Standard Deviation	14.35	65.76	308.48	19.43	76.24	349.79
Consistency (%CV)	4.6	5.7	5.7	5.7	6.9	7.0

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13. Technical Resources

13.1 References

13.1.1 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.

13.1.2 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.

13.1.3 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.

13.2 Technical Support

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