RES108-EN.01

resDetect™ Human Fibronectin ELISA Kit (Residue Testing)

Pack Size: 96 tests

Catalog Number: RES-A108

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedure

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Content

【Background】

Fibronectin, a vital extracellular matrix protein, significantly impacts cell adhesion, spreading, migration, tissue repair, cell shape maintenance, and cell signaling. It exists as a dimer with disulfide - linked polypeptide chains and multiple functional domains. Different isoforms, like soluble and cell - associated ones, play distinct roles in wound healing, blood clotting, and cell - matrix interactions.

To support the development of cell therapy drugs, ACROBiosystems developed Human fibronectin quantitive ELISA Kit with rigorous methodological validation, which is used for the detection and quantitative determination of fibronectin in cell culture supernates, serum, and plasma. Besides, this kit can also be used for the quantitative determination of GMP human fibronectin Protein (ACROBiosystems, cat# GMP-FINH18) concentrations.

【Principle of the assay】

This assay kit is used to measure the levels of human Fibronectin by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-Human Fibronectin Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti-Human Fibronectin Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450 nm and 630 nm. The OD Value reflects the amount of human Fibronectin bound.

【Precautions】

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. Do not use reagents past their expiration date.
3. Do not mix or substitute reagents with those from other kits or other lot number kits.
4. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay.
5. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage. The kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

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【Material provided】

Table 1. Materials provided

ID	Component	Size (96 T)	Format	Storage	
				Unopened	Opened
RES108-C01	Pre-coated Anti-Human Fibronectin Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES108-C02	Human Fibronectin Standard	5 μg×2	Powder	2-8°C	-70°C
RES108-C03	Biotin-Anti-Human Fibronectin Antibody	20 μg	Powder	2-8°C	-70°C
RES108-C04	Streptavidin-HRP	50 μL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES108-C05	10×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES108-C06	2×Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES108-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES108-C08	Stop Solution	7 mL	Liquid	2-8°C	2-8°C

Note: It is recommended that Streptavidin-HRP be centrifuged briefly before use to deposit liquid from the tube wall or cap to the bottom of the tube.

【Storage】

1. Unopened kit should be stored at 2°C-8°C upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per components table. The shelf life is 30 days from the date of opening.

【Reagents and Consumables / Equipment needed but not supplied】

Table 2. Reagents and Consumables / Equipment needed but not supplied

Item	Specification
Deionized or distilled water	/
Single or multi-channel micropipettes	Pipettes must be calibrated
Low retention pipette tips	10 μL, 100 μL, 300 μL, 1000 μL
Reagent bottle	500 mL

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Centrifugal tube	1.5 mL, 10 mL
Microporous plate shaker	/
Vortex	/
Timer	/
Incubator	Can be set at 37℃
Microplate reader	Single or dual wavelength microplate reader capable of measuring signals of 96-well microplates at absorbance wavelengths of 450 nm and 630 nm.

【Quick guide】

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【Reagent preparation】

Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

According to Table 3, prepare the provided lyophilized product into a storage solution with ultrapure water, dissolve at room temperature for 15 to 30 minutes, and mix by gently pipetting, avoiding vigorous shaking or vertexing. The reconstituted storage solution should be stored at -70°C. It is recommended that the number of freezing and thawing should not exceed 1 time, the size of the aliquot should not be less than 5 μg.

Note: Considering inevitable minor quantitation variations between protein batches, it is also reasonable to generate the standard curve with specific lot of proteins used for current production for even better accuracy.

Table 3. Preparation method

ID	Components	Format (96 T)	Concentration	Reconstituted water Vol.
RES108-C02	Human Fibronectin Standard	5 μg	50 μg/mL	100 μL
RES108-C03	Biotin-Anti-Human Fibronectin Antibody	20 μg	200 μg/mL	100 μL

【Recommend sample preparation】

1. Working solution preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-Human Fibronectin Antibody working fluid:

Dilute Biotin-Anti-Human Fibronectin Antibody to 0.2 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

1.5 Sample preparation:

If the sample to be tested is the cell supernatant, dilute test sample at 1:2 with 1×Dilution Buffer. The

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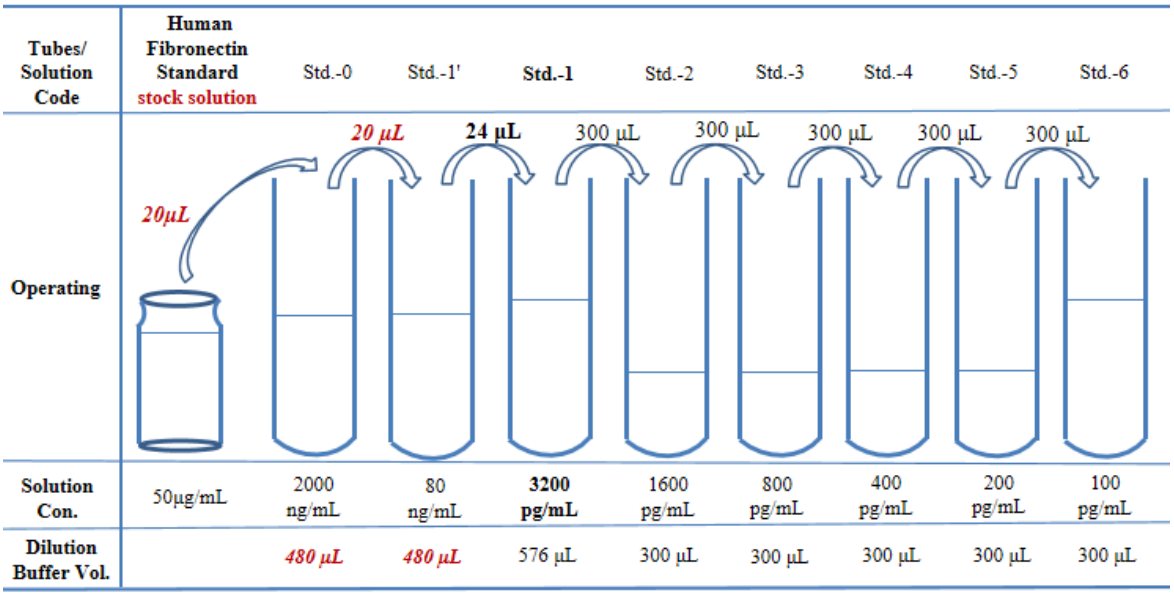
volume ratio of samples to diluent is 1:1.

2. Preparation of standard curve

The concentration of the reconstituted human Fibronectin Calibrator (RES108-C02) is 50 μg/mL, prepare (Std.-0) by diluting 20 μL the reconstituted human Fibronectin Calibrator into 480 μL 1×Dilution Buffer, mix gently well. Then prepare Std.- 1' by diluting 20 μL Std.-0 into 480 μL 1×Dilution Buffer. At last, prepare the highest concentration of standard curve, Std.-1 (3200 pg/mL), by diluting 24 μL Std.- 1' into 576 μL 1×Dilution Buffer. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 μL of 1×Dilution Buffer into each tube. Make sure to mix well every time. 1×Dilution Buffer serves as blank.

Figure 1. Preparation of 1:1 serial dilution of the Human Fibronectin standard



3. Add samples

Add 100 μL Calibrator and samples to each well. For blank Control wells, please add 100 μL 1×Dilution Buffer.

Note: It is recommended to set double holes for samples and standard curves to be tested. Blank is a Blank Dilution Buffer hole.

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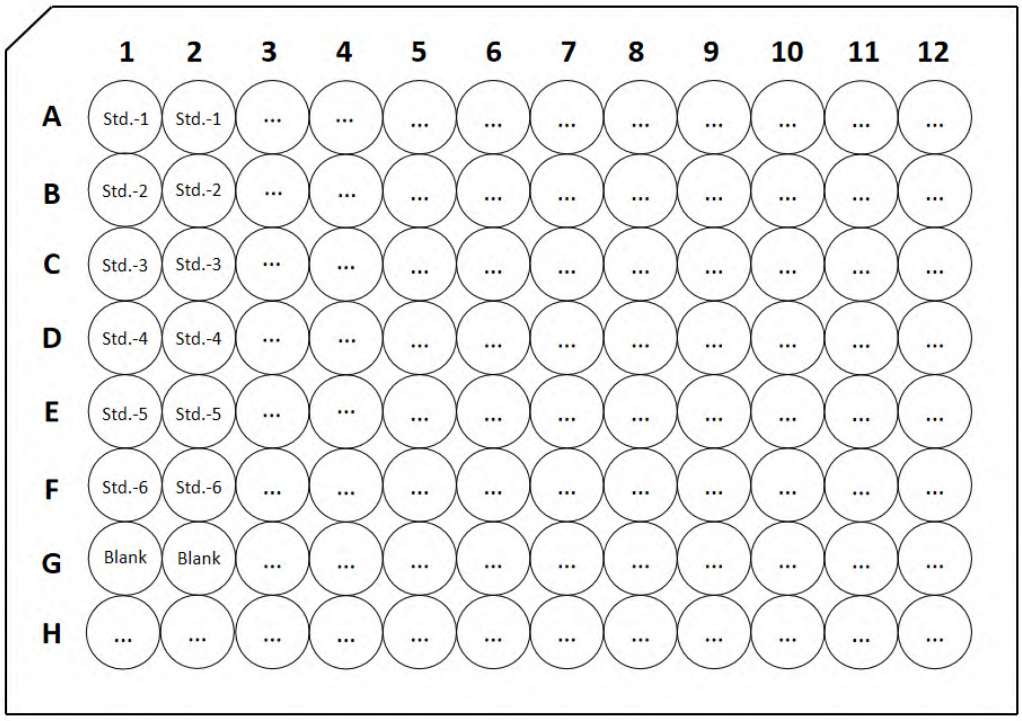
Figure 2. This plate layout is recommended for recording standards and samples



4. Incubation

Seal the plate with microplate sealing film, and incubate at room temperature for 1.0 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10 s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-Human Fibronectin Antibody

For all wells, add 100 µL Biotin-Anti-Human Fibronectin Antibody (dilute to 0.2 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film, and incubate at room temperature for 1.0 hour.

8. Washing

Repeat step 5.

9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

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Seal the plate with microplate sealing film, and incubate at room temperature for 1.0 hour.

11. Washing

Repeat step 5.

12. Substrate reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well and tap the plate gently to allow thorough mixing.

Note: The color in the wells should change from blue to yellow.

14. Data recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 10 minutes.

Note: To reduce the background noise, subtract the value read at OD_{450nm} with the value read at OD_{630nm}.

【Calculation of results】

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (OD).
2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.
3. Normal range of Standard curve: $R^2 \geq 0.9900$.
4. Detection range: 100 pg/mL-3200 pg/mL. If the OD value of the sample to be tested is higher than 3200 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 100 pg/mL, the sample should be reported.

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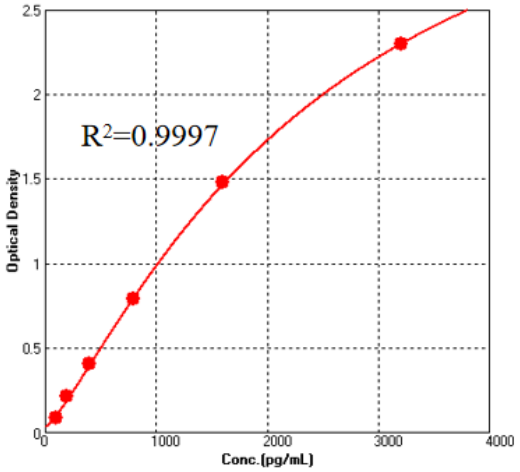
【Typical data】

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only. The sample concentration was calculated based on the results of the standard curve.

Standard (pg/mL)	O.D.-1	O.D.-2	Average	Corrected
3200	2.399	2.303	2.351	2.300
1600	1.548	1.517	1.533	1.482
800	0.847	0.834	0.841	0.790
400	0.473	0.439	0.456	0.405
200	0.277	0.257	0.267	0.216
100	0.147	0.139	0.143	0.092
0	0.051	0.052	0.051	/



【Sensitivity】

The minimum detectable concentration of human Fibronectin is 12.736 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

【Precision】

1. **Intra-assay Precision**

   Three samples of known concentration were tested ten times on one plate to assess intra-assay precision.

2. **Inter-assay Precision**

   Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	10	10	10	3	3	3
Mean (pg/mL)	2265.617	905.105	233.270	2297.290	903.207	236.004
SD	78.964	26.849	6.349	60.611	10.168	5.699
CV (%)	3.5	3.0	2.7	2.6	1.1	2.4

【Recovery】

Five parts of blank MSC cell culture supernatant were added with different concentrations of human Fibronectin, and the MSC cell culture supernatant without human Fibronectin was used as background to calculate the recovery rate. The range of recovery rate is 86.9-102.1%, and the average recovery is 93.8%.

Sample(n=5)	Average Recovery %	Range %
MSC cell culture supernatant (n=5)	93.8	86.9-102.1

【Linearity】

To assess the linearity of the assay, samples spiked with high concentrations of Fibronectin were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture medium (DMEM)	Cell culture medium (1640)
1:2	Average Recovery (%)	91.9	91.8
	Range (%)	88.2-95.1	89.7-95.5
1:4	Average Recovery (%)	96.8	89.6
	Range (%)	89.9-104.2	88.9-90.2
1:8	Average Recovery (%)	96.3	89.1
	Range (%)	91.2-100.2	87.0-91.9
1:16	Average Recovery (%)	96.9	97.3

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	Range (%)	91.6-100.3	86.8-101.3

Note: The example data is for reference only.

【Specificity】

This assay recognizes natural and recombinant human Fibronectin. No cross-reactivity was observed when this kit was used to analyze the following recombinant cytokines.

Human			
TGF-beta 1 Laminin 511 E8	PDGF-BB Activin A	FGF basic	Laminin 521 E8

【Interfering substances】

Verify potential matrix effects by adding different levels of DMSO and HSA to the diluted buffer.

Additive	Tolerated concentration
DMSO	5%
HSA	5%

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【Troubleshooting guide】

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again

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