

# Human Fibronectin ELISA Kit

EHFN1

**Number**

EHFN1

**Description**
**Human Fibronectin ELISA Kit**, sufficient reagents for 96 determinations

| <b>Kit Contents</b>                                  | <b>Size</b> |
|--|-------------|
| Anti-Human Fibronectin Precoated 96-well Strip Plate | 1 each      |
| Lyophilized Recombinant Human Fibronectin Standard   | 2 vials     |
| 20X Wash Buffer                                      | 25mL        |
| Biotinylated Antibody Reagent                        | 2 vials     |
| Streptavidin-HRP Reagent                             | 200uL       |
| TMB Substrate  | 12mL        |
| Stop Solution, contains 0.2M sulfuric acid           | 8mL         |
| Assay Diluent (5X concentrated buffer)               | 15 mL       |
| Adhesive Plate Sealer                                | 2           |

For research use only. Not for use in diagnostic procedures.

**Storage:** May be stored for up to 6 months at 2° to 8°C from the date of shipment. Opened microplate wells or reagents may be store for up to 1 month at 2° to 8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Reconstituted standard can be stored at -80°C for up to 1 week.

**Note:** the kit can be used within one year if the whole kit is stored at -20°C. Avoid repeated freeze-thaw cycles.

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## Introduction

The Thermo Scientific™ Pierce™ Human Fibronectin ELISA Kit is an enzyme-linked immunosorbent assay for measuring human fibronectin in serum, plasma, and cell culture media.

## Procedure Summary

**1.** Prepare all reagents, samples and standards as instructed.



**2.** Add 100µL standard and sample to wells. Cover plate & incubate at RT for 2.5 hours.



**3.** Wash plate four times



**4.** Add 100µL Biotinylated Antibody to wells. Cover plate & incubate at RT for 1 hour.



**5.** Wash plate four times



**6.** Add 100µL of Streptavidin-HRP Reagent to each well.



**7.** Cover & incubate plate at RT for 45 minutes.



**8.** Wash plate four times



**9.** Add 100µL TMB Substrate to each well.



**10.** Develop plate at room temperature in the dark for 30 minutes.



**11.** Add 50µL of Stop Solution to each well.



**12.** Measure absorbance and calculate results

## Additional Materials Required

- Precision pipettors with disposable plastic tips to deliver 5-1000µL and plastic pipettes to deliver 5-15mL
- Ultrapure water for Wash Buffer and Standard reconstitution
- A glass or plastic 1L container to prepare Wash Buffer
- A squirt wash bottle or an automated 96-well plate washer
- 1.5mL polypropylene or polyethylene tubes to prepare standards - do not use polystyrene, polycarbonate or glass tubes
- Disposable reagent reservoirs
- A standard ELISA plate reader for measuring absorbance at 450nm and 550nm. If a 550nm filter is not available, the absorbance may be read at 450nm only. Refer to the instruction manual supplied with the instrument being used.

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## Precautions

- All specimens and reagents must be at room temperature (20-25°C) before use in the assay.
- Review all instructions carefully and verify components against the Kit Contents list (page 1) before beginning the assay.
- Do not use a 37°C water bath to thaw samples. Thaw samples at room temperature.
- If using a multichannel pipettor, always use a new disposable reagent reservoir for the addition of each reagent. Use new disposable pipette tips for each transfer to avoid cross-contamination.
- Use a new adhesive plate cover for each incubation step.
- Avoid microbial contamination of reagents.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Do not mix reagents from different kit lots. Discard unused ELISA components after completing the assay.
- Do not use glass pipettes to measure the TMB Substrate Solution. Take care not to contaminate the solution. If the solution is blue before use, DO NOT USE IT.
- Individual components may contain antibiotics and preservatives. Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedures.
- Some components of this kit contain sodium azide. Please dispose of reagents according to local regulations.

## Sample Preparation

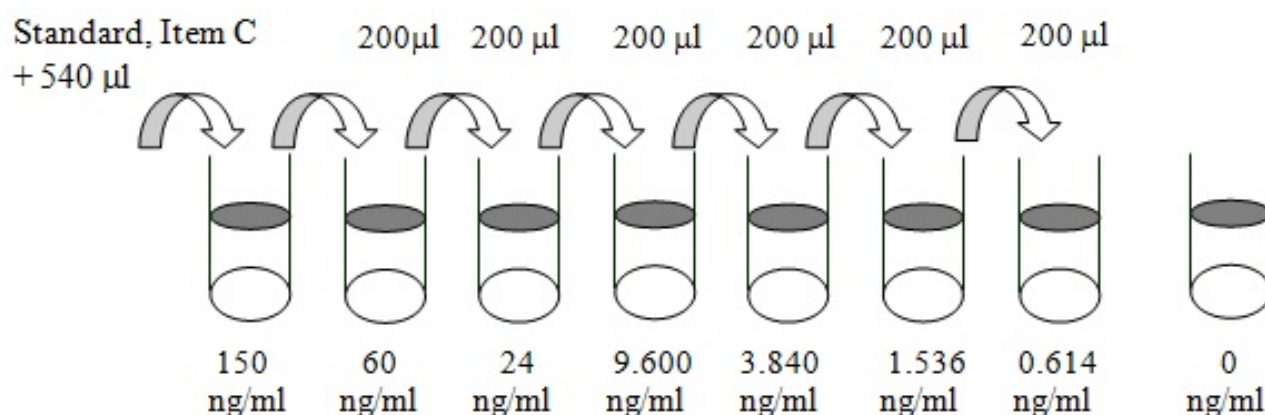
- Serum, plasma, and cell culture media sample types may be tested in this assay; 100µL per well of diluted sample is required. See reagent preparation step 3 for sample dilution recommendations.
- Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles when storing samples.
- Samples and standards must be assayed in duplicate each time the assay is performed.
- Equilibrate samples gradually to room temperature before beginning the assay. Do not use a heated water bath to thaw or warm samples.
- Mix samples by gently inverting the tubes.
- If samples are clotted, grossly hemolyzed, lipemic or contaminated, make a note on the template and interpret results with caution.

## Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25°C) before use.
2. Assay Diluent should be diluted 5-fold with deionized or distilled water before use.
3. **Sample dilution:** 1X Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples. The suggested dilution for normal serum/plasma is 10,000 fold.

**Note:** Levels of Fibronectin may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. **Preparation of standard:** Briefly spin a vial of lyophilized standard. Add 540 µL of 1X Assay Diluent (Assay Diluent should be diluted 5-fold with deionized or distilled water before use) into the lyophilized standard vial to prepare a 150 ng/mL standard. Dissolve the powder thoroughly by gentle mixing. Pipette 300 µL of 1X Assay Diluent into each tube. Use the stock standard solution to produce a dilution series (shown below). Mix each tube thoroughly before the next transfer. 1X Assay Diluent serves as the zero standard (0 ng/mL).



5. If the 20X Wash Buffer contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20mL of the Wash Buffer Concentrate into deionized or distilled water to yield 400mL of 1X Wash Buffer.
6. Briefly spin the Biotinylated Antibody Reagent vial before use. Add 100µL of 1X Assay Diluent into the vial to prepare a biotinylated antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The biotinylated antibody concentrate should be diluted 80-fold with 1X Assay Diluent and used in step 4 of Assay Procedure.
7. Briefly spin the Streptavidin-HRP Reagent vial and pipette up and down to mix gently before use, as precipitates may form during storage. The Streptavidin-HRP Reagent should be diluted 1,300-fold with 1X Assay Diluent.

For example: Briefly spin the vial and pipette up and down to mix gently. Add 10µL of HRP-Streptavidin concentrate into a tube with 13mL 1X Assay Diluent to prepare a 1,300-fold diluted HRP-Streptavidin solution (do not store the diluted solution for next day use). Mix well.

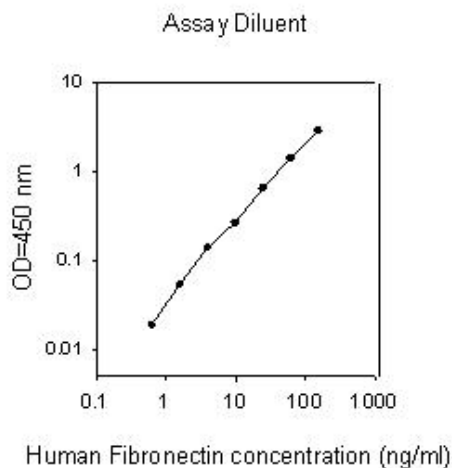
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## Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100µL of each standard (see Reagent Preparation step 3) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.  
  
**Note:** Overnight incubations at 4°C with gentle shaking can be performed, but may increase overall signals including background.
3. Discard the solution and wash 4 times with 1X Wash Buffer. Wash by filling each well with Wash Buffer (300µL) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100µL of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100µL of prepared Streptavidin-HRP solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add 100µL of TMB Substrate to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 µl of Stop Solution to each well.
10. The plate must be evaluated within 30 minutes of stopping the reaction. Measure absorbance on an ELISA plate reader set at 450nm and 550nm. Subtract 550nm values from 450nm values to correct for optical imperfections in the microplate. If 550nm is not available, measure absorbance at 450nm only. Omitting the 550nm measurement will result in higher absorbance values.
11. Calculation of Results:
  - Generate the standard curve by plotting the average absorbance (450nm minus 550nm) obtained for each Standard concentration on the vertical (Y) axis vs. the corresponding Fibronectin concentration on the horizontal (X) axis.
  - Calculate results manually using graph paper or with a curve-fitting statistical software package. If using curve-fitting software, plot a four-parameter logistic curve fit. Alternatively, a point-to-point curve fit may be used. Determine the amount of Fibronectin in each sample by interpolating from the Fibronectin concentration (X axis) to the absorbance value (Y axis).
  - If the sample was diluted, multiply the interpolated value obtained by the dilution factor to determine amount of Fibronectin in the sample.
  - Absorbance values obtained for duplicates should be within 10% of the mean value. Carefully consider duplicate values that differ from the mean by greater than 10%.

## Performance Characteristics

**Typical Data:** These standard curves are for demonstration only. A standard curve must be run with each assay.



**Sensitivity:** 0.6 ng/mL

The sensitivity or Lower Limit of Detection (LLD) was determined by assaying replicates of zero and the standard curve. The mean signal of zero + 2 standard deviations read in dose from the standard curve is the LLD. This value is the smallest dose that is not zero with 95% confidence.

**Spiking & Recovery:** Pooled serum, plasma, and cell culture media samples were spiked with recombinant Human Fibronectin. Endogenous Human Fibronectin levels were determined by testing non-spiked samples alongside spiked aliquots of the same samples. Expected values were calculated by adding endogenous Fibronectin levels to those of the spiked control. Percent recovery was calculated by dividing observed by expected values.

| Sample Type        | Average % Recovery | Range (%) |
|--------------------|--------------------|-----------|
| Serum              | 93                 | 80-105    |
| Plasma             | 82                 | 67-93     |
| Cell Culture Media | 72                 | 68-82     |

**Linearity:** The serum, plasma, and cell culture media samples were spiked with recombinant Human Fibronectin, serially diluted in sample diluent and evaluated. Observed values were compared to expected values to calculate percent recovery and demonstrate the dilution linearity of the assay.

| Sample Type        | Average % Expected |              | Range (%)    |              |
|--------------------|--------------------|--------------|--------------|--------------|
|                    | 1:2 Dilution       | 1:4 Dilution | 1:2 Dilution | 1:4 Dilution |
| Serum              | 89                 | 73           | 83-94        | 69-76        |
| Plasma             | 75                 | 71           | 68-83        | 69-74        |
| Cell Culture Media | 81                 | 83           | 76-86        | 78-87        |








**Reproducibility:** Assay reproducibility was evaluated in each sample matrix. To determine intra-assay precision, two standard curves and 3 samples for each standard curve are run. The standard curve concentration points as well as the samples are tested in duplicates on a single plate. Two different concentration values are obtained for each sample, using the two separate standard curves. The two concentration values for each sample is compared to each other using the CV% calculation. To evaluate inter-assay precision, the second standard curve is tested on a separate plate along with the second set of samples.

Intra-Assay CV%: <10%

Inter-Assay CV%: <12%

**Specificity:** This ELISA pair antibody detects human Fibronectin. Other species not determined.

#### Explanation of symbols

| Symbol  | Description                  | Symbol  | Description                             |
|---|------------------------------|---|---|
|  | Manufacturer                 |  | Catalog number                          |
|  | Use by                       |  | Temperature limitation                  |
|  | Consult instructions for use |  | Caution, consult accompanying documents |
|  | Batch code                   |   |   |

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**Data Templates**

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |

|   | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|----|----|----|
| A |   |   |   |   |   |   |   |   |   |    |    |    |
| B |   |   |   |   |   |   |   |   |   |    |    |    |
| C |   |   |   |   |   |   |   |   |   |    |    |    |
| D |   |   |   |   |   |   |   |   |   |    |    |    |
| E |   |   |   |   |   |   |   |   |   |    |    |    |
| F |   |   |   |   |   |   |   |   |   |    |    |    |
| G |   |   |   |   |   |   |   |   |   |    |    |    |
| H |   |   |   |   |   |   |   |   |   |    |    |    |