Introduction

The adhesive glycoprotein fibronectin (FN), Mr440,000, is widely distributed on cell surfaces, in the extracellular matrix, and in plasma. FN is involved in a variety of cellular processes, including cell-to-substrate adhesion, cell migration, and regulation of cell morphology (1). Webb et al first reported in 1980 (2) that the amount of fragmented FN in the urine of patients with prostatic cancer was increased compared to normal subjects. Another group has reported that an FN-like protein, with a low molecular mass than plasma FN, was increased in sera from patients with malignant disease (3). In particular, many examinations have been done to evaluate the clinical usefulness of measurements of plasma FN (4-7). Recently, Katayama et al reported that urinary FN (UFN) in cancer patients was almost always present as fragments of different sizes containing the central “cell-binding domain”, and that a polyclonal antibody raised against native plasma FN could not react well with these fragmented UFN. They demonstrated that two monoclonal antibodies, reactive with the “cell-binding domain” (8) are suitable for in vitro assays of FN in biological fluids and FN-fragments excreted in the urine of cancer patients, and that significantly higher levels of UFN were observed in many kinds of cancer (9,10).
**Intended use**
The human Fibronectin EIA Kit is an *in vitro* enzyme immunoassay (EIA) kit for the specific quantitative determination of human Fibronectin (hFN) in serum, urine, cell culture supernatants, and other biological fluids. This kit is suitable for quantitation of soluble human Fibronectin. *This kit is for research use only. It is not for use in diagnostic or therapeutic procedures.*

**Principle**
The human Fibronectin EIA Kit is a solid phase EIA based on a sandwich method that utilizes two mouse monoclonal anti-human Fibronectin antibodies to detect Fibronectin by two-step procedure. One of the mouse monoclonal anti-human Fibronectin antibodies is bound to a microtiter plate to create the solid phase. Non-specific binding is blocked by a blocking buffer. Samples and standards are incubated in microtiter-plate wells. The second step is to wash the plate and add a second anti-Fibronectin antibody labelled with peroxidase (POD). During the incubation, human Fibronectin, bound to anti-human Fibronectin antibody (solid phase) on one side, is tagged on the other by POD-anti-human Fibronectin antibody. The reaction between POD and substrate ($H_2O_2$ and tetramethyl benzidine) results in colour development with intensities proportional to the amount of human Fibronectin present in samples and standards. The amount of human Fibronectin can be quantitated by measuring the absorbance using an EIA plate reader. Accurate sample concentrations of human Fibronectin can be determined by comparing their specific absorbances with those obtained for the standards plotted on a standard curve.
Reagents and materials
Each human Fibronectin EIA Kit includes reagents sufficient for 96 wells. The expiration date for the complete kit is stated on the outer box label and the recommended storage temperature is 2 - 8°C.

A. Materials provided
Plate 1. Antibody Coated Microtiterplate - 1 plate (8 well x 12 strips)
The plate coated with murine monoclonal antibody to human Fibronectin
Store at 2 - 8°C.

Vial 2. Antibody-POD Conjugate - 1 vial (for 11 ml x 1)
The vial contains lyophilized horseradish peroxidase (POD) conjugated murine monoclonal antibody to human and bovine Fibronectin.
Store at 2 - 8°C. Avoid prolonged exposure to light.

Vial 3. Standard - 1 vial (800 ng x 1)
The vial contains lyophilized human Fibronectin Fragment (cell binding domain)

Vial 4. Sample Diluent - 2 vials (11 ml x 2)
Each vial contains protein in a buffered solution. Use for Zero standard, and for dilution of the standard (vial 3) and samples which are above the calibration curve. Store at 2 - 8°C.

Vial 5. Substrate Solution- 1 vial (12 ml x 1)
Each vial contains hydrogen peroxide and tetramethylbenzidine in a buffered solution. Store at 2-8°C.

B. Materials required but not provided
Reagents
- Stop Solution: 1N H₂SO₄
- Washing Buffer: Phosphate-buffered Saline (PBS) containing 0.1% Tween 20
  (PBS tablets (Cat.#T900) is convenient for the preparation of Washing Buffer.)

Materials
- Precision pipettes with disposable tips: 20 and 100 µl micropipettes,
- 10 - 200 µl adjustable multiwell pipetter or 100 µl multiwell pipetters
- Beakers, flasks, cylinders necessary for preparation of reagents
- Disposable pipettes and test tubes
- Microtiter plate reader for measurement of absorbance at 450 nm
- Graph paper
Precautions
- Do not mix reagents from different kit lots.
- Do not use reagents beyond expiration date on label.
- In order to avoid reagent contamination, use disposable pipette tips and/or pipettes.
- Sodium azide inactivates POD. Solutions containing sodium azide should not be used in this assay.
- Do not expose Substrate Solution to strong light during storage or incubation.
- Avoid contact of Substrate and Stop Solution with skin or mucous membranes. If these reagents come into contact with skin, wash thoroughly with water. Do not pipette by mouth. Do not smoke, eat, or drink in area where specimens or kit reagents are handled. All blood fluids should be considered as potentially infectious.
- Avoid contact of Substrate Solution and Stop Solution with any metal surface. Disposable glassware or test tubes are recommended for handling the Substrate Solution. If non-disposable glassware is used, it must be acid washed and thoroughly rinsed with distilled, deionized water.
- Do not use the Substrate Solution if its colour is changed to thick blue.

Specimen collection and handling
Plasma, serum, urine or cell culture supernatant is suitable for use in the assay, however, cell or tissue extract can be also used. PBS containing 0.1% NaN₃, 5 mM EDTA and 0.3 mM Phenylmethylsulfonyl fluoride (pH7.2) should be used for preparation of cell extracts. Remove the serum from the clot or red cells, respectively, soon after clotting and separation. Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens. Plasma and serum samples must be diluted several hundred fold. Urine samples can be used directly, but it is necessary to be adjusted by urine creatinine as ng FN/mg creatinine for the data comparison. Samples may be stored up to 24 hours at 4°C. If the length of time between sample collection and assay is to exceed 24 hours, samples should be stored frozen under -20°C for optimal results. Excessive freeze-thaw cycles should be avoided. Prior to assay, frozen samples should be brought to room temperature slowly, and gently mixed by hand. Do not thaw samples in a hot bath. Do not vortex or sharply agitate.

Recommended Sample Dilution
In case of using plasma or serum, dilute the samples by 100-250 folds before assay. In case of using urine, no need to dilute the sample before assay. When the diluted samples generate values out of the standard range, dilute the samples with the different dilution rate referring to the first assay result, and repeat the assay. Or it is recommended to assay using three kinds of sample dilutions making the 100-250 folds as the middle concentration. In case of using urine samples, they can be used directly.
Preparation of solutions

Note: The following solutions should be prepared directly before use.

Solution 1. Antibody-POD Conjugate Solution
Dissolve the contents of Vial 2 in 11 ml of distilled water and mix gently followed by 10 minutes slowly rolling or occasional mixing, avoiding foam formation.

Solution 2. Standard Solution
Rehydrate Standard (Vial 3) with 1 ml of distilled water. Slowly roll for approximately 10 minutes or let the vial stand and sporadically mix gently.

The standard solution contains 800 ng human Fibronectin/ml.

Prepare dilution series of 400, 200, 100, 50, 25 and 12.5 ng/ml by diluting the Standard Solution with Sample Diluent.

Stability of solutions

Solution 1. Antibody-POD Conjugate Solution
The reconstituted lyophilisate is stable for 1 week at 4°C and for 1 month when stored at -20°C. Do not repeat freeze-thaw cycles.

Solution 2. Standard Solution
The reconstituted lyophilisate is stable for 2 weeks stored at -80°C. Do not repeat freeze-thaw cycles.

Procedure
Double determinations of all samples and standards should be performed. All of the Kit’s content should be brought to room temperature before use!
For thorough mixing, the microtiter plate can be gently agitated on a plate mixer or by mixing the plate sporadically by hand.

[ Enzyme Immunoassay ]
- Sample incubation: Pipette 100 μl of sample or standard (Solution 2) into one well within 5 minutes. Mix, seal the microtiter plate (e.g. with a foil) and incubate for 1 hour at 37°C.
- Remove sample solution and wash the wells 3 times with ca. 400 μl of Washing Buffer; between the separate washing steps, empty out the microtiter plate and vigorously tap onto paper towel, especially after the last washing.
- Antibody-POD conjugate incubation: Pipette 100 μl of Antibody-POD conjugate Solution (Solution 1) into one well. Mix, seal the microtiter plate (e.g. with a foil) and incubate 1 hour at 37°C.
- Remove the sample solution and wash the wells 4 times as described above (It is specially important after this step to thoroughly empty out the remaining fluid before adding the substrate).
- Substrate incubation: Add 100 μl of Substrate Solution (Vial 5) into each well and incubate at room temperature (20-30°C) for 15 minutes.
- Add 100 μl of Stop Solution (1N H₂SO₄) into each well in the same order as for substrate. Tap plate gently to mix.
- Measure the absorbance at 450 nm with a plate reader. The absorbance should be read as soon as possible after the completion of the assay. It may be read up to 1 hour after addition of Stop Solution if wells are protected from light at room temperature.

  Note: It is important that Stop Solution is added to wells prior to reading at 450 nm. Addition of Stop Solution causes an increase in absorbance of the Substrate Solution and shift in absorbance spectrum.

Results
1. Standard curve
   - Record the absorbance at 450 nm for each standard well.
   - Average the duplicate values and record the averages.
   - Plot the absorbance (vertical axis) versus the Fibronectin concentration in ng/ml (horizontal axis) for the standards using optimal fitting curve.

2. Samples
   - Record the absorbance at 450 nm for each sample well.
   - Average the duplicate values and record the averages.
   - Locate the average absorbance value on the vertical axis and follow a horizontal line intersecting the standard curve. At the point of intersection, read the Fibronectin concentration (ng/ml) from the horizontal axis.

Performance characteristics
1. Range of standard curve: 12.5-800 ng/ml.
2. Specificity: This kit specifically measures human Fibronectin. This kit cannot be used to measure mouse, bovine, rabbit, porcine Fibronectin. The application of this kit for quantitating Fibronectin from other sources has not been tested.
3. Assay duration: Two and a half hour after sample incubation
4. Total assay capacity: 96 assays
5. Assay capacity for test samples: If all assay wells (including standards and test samples) are run in duplicate, 40 test samples can be run in duplicate per kit.
6. Test specimen type: Human plasma, serum and urine; culture supernatants,
7. Specimen volume required: If each test sample is run in duplicate, approximately 220 μl (i.e., 100 μl per assay well plus ~10 μl for each sample transfer) is required.
8. Limitation: Since conditions may vary from assay to assay, a standard curve must be established for every run. Since cross contamination between reagents will invalidate the test, disposable pipette tips should be used.

Thorough washing of the wells between incubations is required:
1) Completely empty out the remaining fluid from the well before dispensing fresh wash solution.
2) Use sufficient wash solution for each wash cycle (approximately 400 μl).
3) Do not allow wells to sit uncovered for extended periods between incubation steps.

Only samples with absorbance values falling within the range of the standard curve should be assigned a human Fibronectin concentration from the curve.
9. **Notes**: According to the assay results using control serum, it could be possible to determine the concentration of antigen present in biological fluids. However, the measurement may be potentially disturbed by the unknown organic factors in serum samples in patients with specific diseases. Similarly, a specimen obtained from an apparent healthy subject might also be interrupted. When an antigen level in an unknown organic specimen is observed to be elevated as compared to the calibration range of the standard curve, it is recommended to dilute the specimens properly with the Sample Diluent (Vial 4) included in the kit and assay them again in another run.

**Basal data**

1. **Typical Standard Curve**

   Each laboratory should establish its own normal range for Fibronectin level.

   (Do Not Use To Calculate Unknowns)

\[
\begin{align*}
\text{Curve Fit: } & 4\text{-Parameter} \\
\text{Corr. Coeff: } & -1.00 \\
y &= \frac{(A-D)}{(1 + (x/C)^B)} + D \\
A &= 0.0536 \quad B = 1.61 \quad C = 707. \quad D = 4.21 
\end{align*}
\]

<table>
<thead>
<tr>
<th>Fibronectin (ng/ml)</th>
<th>800</th>
<th>400</th>
<th>200</th>
<th>100</th>
<th>50</th>
<th>25</th>
<th>12.5</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_{450})</td>
<td>2.336</td>
<td>1.242</td>
<td>0.530</td>
<td>0.226</td>
<td>0.115</td>
<td>0.079</td>
<td>0.058</td>
<td>0.046</td>
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</tbody>
</table>
2. Intra-assay precision (n=16)
Assay was carried out with 16 replicates of 3 samples containing different concentration of control Fibronectin. All samples was diluted 100-fold for assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ave. (ng/ml)</th>
<th>S.D.</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>244.4</td>
<td>9.49</td>
<td>3.9</td>
</tr>
<tr>
<td>Control B</td>
<td>61.4</td>
<td>2.87</td>
<td>4.7</td>
</tr>
<tr>
<td>Control C</td>
<td>22.7</td>
<td>1.61</td>
<td>7.1</td>
</tr>
</tbody>
</table>

Inter-assay precision (performance 3 times)
Assay to assay precision with one laboratory was evaluated in 3 independent experiments over 3 days. All samples was diluted 100-fold for assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Ave. (ng/ml)</th>
<th>S.D.</th>
<th>CV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control A</td>
<td>412.9</td>
<td>12.91</td>
<td>3.1</td>
</tr>
<tr>
<td>Control B</td>
<td>124.0</td>
<td>7.43</td>
<td>6.0</td>
</tr>
<tr>
<td>Control C</td>
<td>51.3</td>
<td>1.87</td>
<td>3.6</td>
</tr>
</tbody>
</table>

3. Recovery test
The recovery of Fibronectin was tested by adding 2 samples out of 5 different level in various matrices.

\[
\text{Recovery(\%)} = \left( \frac{\text{A+B measured}}{\text{A+B calculated}} \right) \times 100
\]

<table>
<thead>
<tr>
<th>Sample A</th>
<th>Sample B</th>
<th>A+B Measured</th>
<th>A+B Calculated</th>
<th>Recovery(%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>589.0</td>
<td>589.0</td>
<td>600.4</td>
<td>589.0</td>
<td>101.9</td>
</tr>
<tr>
<td>589.0</td>
<td>316.7</td>
<td>445.4</td>
<td>452.9</td>
<td>98.4</td>
</tr>
<tr>
<td>589.0</td>
<td>161.8</td>
<td>395.2</td>
<td>375.4</td>
<td>105.3</td>
</tr>
<tr>
<td>589.0</td>
<td>85.6</td>
<td>338.8</td>
<td>337.3</td>
<td>100.4</td>
</tr>
<tr>
<td>589.0</td>
<td>39.5</td>
<td>331.3</td>
<td>314.2</td>
<td>105.4</td>
</tr>
<tr>
<td>589.0</td>
<td>0.0</td>
<td>255.1</td>
<td>294.5</td>
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<tr>
<td>316.7</td>
<td>316.7</td>
<td>309.2</td>
<td>316.7</td>
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</tr>
<tr>
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<tr>
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<td>200.2</td>
<td>201.2</td>
<td>99.5</td>
</tr>
<tr>
<td>316.7</td>
<td>39.5</td>
<td>175.0</td>
<td>178.1</td>
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</tr>
<tr>
<td>316.7</td>
<td>0.0</td>
<td>151.0</td>
<td>158.4</td>
<td>95.4</td>
</tr>
<tr>
<td>161.8</td>
<td>161.8</td>
<td>151.7</td>
<td>161.8</td>
<td>93.8</td>
</tr>
<tr>
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<td>119.8</td>
<td>123.7</td>
<td>96.8</td>
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<tr>
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<td>39.5</td>
<td>109.0</td>
<td>100.6</td>
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<td>80.9</td>
<td>99.1</td>
</tr>
<tr>
<td>85.6</td>
<td>85.6</td>
<td>76.4</td>
<td>85.6</td>
<td>89.3</td>
</tr>
<tr>
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<td>39.5</td>
<td>58.6</td>
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<td>93.7</td>
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<tr>
<td>85.6</td>
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<td>37.7</td>
<td>42.8</td>
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<tr>
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<tr>
<td>39.5</td>
<td>0.0</td>
<td>19.8</td>
<td>19.7</td>
<td>100.5</td>
</tr>
</tbody>
</table>

* \[\text{Recovery(\%)} = \left( \frac{\text{A+B measured}}{\text{A+B calculated}} \right) \times 100\]
4. Dilution curves of serum samples
Each assay was performed using 3 kinds of samples which were prepared as dilution series starting from 100-fold.

![Graph showing dilution curves of serum samples.](image)

```
No.1:  y = 2.91 \times 10^{4} x + 14.2  \quad r = 0.992 
No.2:  y = 1.71 \times 10^{4} x + 14.8  \quad r = 0.996 
No.3:  y = 2.65 \times 10^{4} x + 11.8  \quad r = 0.998 
```

5. Epitope of the antibodies supplied in this kit
Solid antibody (Antibody coated on the plate): FN30-8 (Cat.#M010)
Labelled antibody (Antibody-POD conjugate): FN12-8 (Cat.#M002)

**Domain Structure**
1: Fibrin-heparin
2: Collagen
3: Heparin
4: Cell SAS: Synergistic adhesion site
5: Heparin RGDS: Arg-Gly-Asp-Ser-peptide
6: Fibrin CS: Connecting segment

**Specificity**

<table>
<thead>
<tr>
<th>Clone No.</th>
<th>Domain Specificity*</th>
<th>Inhibition of Cell adhesion</th>
<th>Cross with bovine FN</th>
<th>Cross with porcine FN</th>
<th>Cross with rabbit FN</th>
<th>Cross with mouse FN</th>
</tr>
</thead>
<tbody>
<tr>
<td>FN12-8</td>
<td>cell</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
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<td>FN30-8</td>
<td>cell</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

(*Please refer to the above figure for Domain Specificity.*)
6. Daily variation of urinary FN and other proteins
The daily variation of urinary Fibronectin, Laminin and E-cadherin was measured using the samples from 3 individuals. For Fibronectin(FN) and Laminin(LN) assay, the samples were used directly. For E-cadherin assay, the samples were used after 9-fold dilution. The following table shows the estimated E-cadherin values at the original concentration. The samples were collected freely during daytime. (This data was obtained by using #MK007 and the former kits #MK015 and #MK017. The value of creatinine was also measured.)

<table>
<thead>
<tr>
<th></th>
<th>FN (ng/ml)</th>
<th>Cr (g/l)</th>
<th>UFN(FN/Cr) (µg/g.Cr)</th>
<th>ULN (LN/Cr) (µg/g.Cr)</th>
<th>UEcad(Ecad/Cr) (mg/g.Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>97.1</td>
<td>1.264</td>
<td>76.8</td>
<td>59.8</td>
<td>2.82</td>
</tr>
<tr>
<td>day 2</td>
<td>66.5</td>
<td>1.004</td>
<td>66.2</td>
<td>42.3</td>
<td>3.66</td>
</tr>
<tr>
<td>day 3</td>
<td>45.6</td>
<td>0.605</td>
<td>75.4</td>
<td>59.7</td>
<td>4.33</td>
</tr>
<tr>
<td>day 4</td>
<td>203.5</td>
<td>1.514</td>
<td>134.4</td>
<td>62.6</td>
<td>2.69</td>
</tr>
<tr>
<td>day 5</td>
<td>135.0</td>
<td>2.346</td>
<td>57.5</td>
<td>34.8</td>
<td>3.42</td>
</tr>
<tr>
<td>day 6</td>
<td>93.7</td>
<td>1.915</td>
<td>48.9</td>
<td>48.6</td>
<td>4.20</td>
</tr>
<tr>
<td>day 7</td>
<td>131.1</td>
<td>1.313</td>
<td>99.8</td>
<td>66.5</td>
<td>3.89</td>
</tr>
<tr>
<td>day 8</td>
<td>51.5</td>
<td>0.483</td>
<td>106.6</td>
<td>127.2</td>
<td>6.08</td>
</tr>
<tr>
<td>2. female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>23.3</td>
<td>1.182</td>
<td>19.7</td>
<td>34.7</td>
<td>1.11</td>
</tr>
<tr>
<td>day 2</td>
<td>35.9</td>
<td>2.045</td>
<td>17.6</td>
<td>31.4</td>
<td>0.59</td>
</tr>
<tr>
<td>day 3</td>
<td>17.0</td>
<td>1.594</td>
<td>10.7</td>
<td>19.0</td>
<td>0.49</td>
</tr>
<tr>
<td>day 4</td>
<td>33.5</td>
<td>1.565</td>
<td>21.4</td>
<td>42.7</td>
<td>0.20</td>
</tr>
<tr>
<td>day 5</td>
<td>16.5</td>
<td>1.814</td>
<td>9.1</td>
<td>26.7</td>
<td>0.40</td>
</tr>
<tr>
<td>day 6</td>
<td>51.5</td>
<td>2.562</td>
<td>20.1</td>
<td>24.8</td>
<td>0.22</td>
</tr>
<tr>
<td>day 7</td>
<td>15.1</td>
<td>2.031</td>
<td>7.4</td>
<td>27.4</td>
<td>0.23</td>
</tr>
<tr>
<td>day 8</td>
<td>23.3</td>
<td>1.579</td>
<td>14.8</td>
<td>38.5</td>
<td>0.33</td>
</tr>
<tr>
<td>3. male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day 1</td>
<td>69.9</td>
<td>1.195</td>
<td>58.5</td>
<td>45.4</td>
<td>0.73</td>
</tr>
<tr>
<td>day 2</td>
<td>0.0</td>
<td>0.403</td>
<td>0.0</td>
<td>23.9</td>
<td>0.17</td>
</tr>
<tr>
<td>day 3</td>
<td>57.3</td>
<td>1.016</td>
<td>56.4</td>
<td>60.5</td>
<td>0.86</td>
</tr>
<tr>
<td>day 4</td>
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<td>1.397</td>
<td>78.9</td>
<td>45.8</td>
<td>0.93</td>
</tr>
<tr>
<td>day 5</td>
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<td>0.422</td>
<td>5.7</td>
<td>39.9</td>
<td>1.37</td>
</tr>
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<td>day 6</td>
<td>10.2</td>
<td>0.561</td>
<td>18.2</td>
<td>77.2</td>
<td>0.14</td>
</tr>
<tr>
<td>day 7</td>
<td>31.6</td>
<td>1.466</td>
<td>21.6</td>
<td>36.4</td>
<td>1.17</td>
</tr>
<tr>
<td>day 8</td>
<td>27.7</td>
<td>0.645</td>
<td>42.9</td>
<td>81.4</td>
<td>1.61</td>
</tr>
</tbody>
</table>
7. Urinary Fibronectin excretion in a day

The amount of urinary Fibronectin and other proteins (LN and E-cadherin) excretion in a day was measured with the samples collected from 4 individuals. For FN and LN assay, the samples were used directly. For E-cadherin assay, the samples were used after 9-fold dilution. In the following table, the estimated E-cadherin values at the original concentration are shown. The samples were collected freely during a day.

(This data was obtained by using #MK007 and the former kits #MK015 and #MK017. The value of creatinine was also measured.)

<table>
<thead>
<tr>
<th></th>
<th>Urine (ml)</th>
<th>FN (ng/ml)</th>
<th>Cr (g/l)</th>
<th>UFN(FN/Cr) (µg/g.Cr)</th>
<th>ULN(LN/Cr) (µg/g.Cr)</th>
<th>UEcad(/Cr) (mg/g.Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. male</td>
<td>270</td>
<td>199.6</td>
<td>1.760</td>
<td>113.4</td>
<td>65.2</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>525</td>
<td>189.9</td>
<td>1.100</td>
<td>172.6</td>
<td>49.3</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>66.5</td>
<td>1.489</td>
<td>44.7</td>
<td>68.3</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>570</td>
<td>11.2</td>
<td>0.897</td>
<td>12.5</td>
<td>40.1</td>
<td>0.47</td>
</tr>
<tr>
<td>5. female</td>
<td>80</td>
<td>17.5</td>
<td>1.331</td>
<td>13.1</td>
<td>62.6</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>40.3</td>
<td>1.441</td>
<td>28.0</td>
<td>93.7</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>22.3</td>
<td>1.478</td>
<td>15.1</td>
<td>40.4</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>45.6</td>
<td>0.974</td>
<td>46.8</td>
<td>54.2</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>38.4</td>
<td>1.466</td>
<td>26.2</td>
<td>53.1</td>
<td>0.63</td>
</tr>
<tr>
<td>6. female</td>
<td>350</td>
<td>23.8</td>
<td>0.584</td>
<td>40.8</td>
<td>38.7</td>
<td>0.85</td>
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<tr>
<td></td>
<td>470</td>
<td>11.7</td>
<td>0.481</td>
<td>24.3</td>
<td>24.6</td>
<td>1.32</td>
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<tr>
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<td>370</td>
<td>12.1</td>
<td>0.676</td>
<td>17.9</td>
<td>41.0</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>76.7</td>
<td>1.548</td>
<td>49.5</td>
<td>40.4</td>
<td>0.50</td>
</tr>
<tr>
<td></td>
<td>130</td>
<td>30.1</td>
<td>1.890</td>
<td>15.9</td>
<td>41.9</td>
<td>0.31</td>
</tr>
<tr>
<td>7. male</td>
<td>250</td>
<td>60.7</td>
<td>1.718</td>
<td>35.3</td>
<td>55.1</td>
<td>1.04</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>148.6</td>
<td>2.759</td>
<td>53.9</td>
<td>42.7</td>
<td>1.47</td>
</tr>
<tr>
<td></td>
<td>160</td>
<td>114.6</td>
<td>1.579</td>
<td>72.6</td>
<td>56.5</td>
<td>2.43</td>
</tr>
<tr>
<td></td>
<td>175</td>
<td>141.8</td>
<td>2.400</td>
<td>59.1</td>
<td>68.3</td>
<td>1.43</td>
</tr>
<tr>
<td></td>
<td>175</td>
<td>95.2</td>
<td>1.844</td>
<td>51.6</td>
<td>64.3</td>
<td>1.33</td>
</tr>
</tbody>
</table>
8. Fibronectin in cell culture supernatant

The amount of Fibronectin in the supernatant of various cells cultured in 10% FCS/RPMI1640 or serum free/Ultradoma PF was measured. The supernatant was applied to assay without dilution. Fetal Calf Serum does not inhibit this assay system. (This data was obtained by using this precoated kit #MK115.)

<table>
<thead>
<tr>
<th>Cell Strain</th>
<th>FN (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HepG2(SF)</td>
<td></td>
</tr>
<tr>
<td>HUVEC(SF)</td>
<td></td>
</tr>
<tr>
<td>HT1080(SF)</td>
<td></td>
</tr>
<tr>
<td>Colo205(SF)</td>
<td></td>
</tr>
<tr>
<td>HL60(SF)</td>
<td></td>
</tr>
<tr>
<td>T24(SF)</td>
<td></td>
</tr>
<tr>
<td>NSK(SF)</td>
<td></td>
</tr>
<tr>
<td>MG63(SF)</td>
<td></td>
</tr>
<tr>
<td>HGH(SF)</td>
<td></td>
</tr>
<tr>
<td>SQ5(SF)</td>
<td></td>
</tr>
<tr>
<td>A431(SF)</td>
<td></td>
</tr>
<tr>
<td>Lu65(SF)</td>
<td></td>
</tr>
<tr>
<td>OST(SF)</td>
<td></td>
</tr>
<tr>
<td>BUD-8(SF)</td>
<td></td>
</tr>
<tr>
<td>KM101(SF)</td>
<td></td>
</tr>
<tr>
<td>KM101(+S)</td>
<td></td>
</tr>
<tr>
<td>SIRC(SF)</td>
<td></td>
</tr>
<tr>
<td>SIRC(+S)</td>
<td></td>
</tr>
<tr>
<td>D-17(SF)</td>
<td></td>
</tr>
<tr>
<td>D-17(+S)</td>
<td></td>
</tr>
<tr>
<td>FLamnion(SF)</td>
<td></td>
</tr>
<tr>
<td>FLamnion(+S)</td>
<td></td>
</tr>
<tr>
<td>N.F.(SF)</td>
<td></td>
</tr>
<tr>
<td>N.F.(+S)</td>
<td></td>
</tr>
<tr>
<td>CPAE(SF)</td>
<td></td>
</tr>
<tr>
<td>CPAE(+S)</td>
<td></td>
</tr>
</tbody>
</table>

+S: 10% FCS/RPMI1640
SF: serum free medium (2 days after the change from the medium containing serum)
9. Influence of coexistence
The volume ratio of sample to co-existing substance is 4:1. Co-existing substance is shown in its final concentration.

- Heparin (mg/ml)
- EDTA.4NA (mg/ml)
- Citrate.2Na (mg/ml)
- Human IgG (mg/ml)
- Human Bilirubin (mg/ml)
- Human Albumin (mg/ml)
- Human Fibrinogen (mg/ml)
- L-ascorbic acid (mg/ml)
- Calcium Chloride (mg/ml)
- Human Hemoglobin (mg/ml)
10. Correlation with the former kit (Takara Cat.#MK015)

Correlation of precoated type kit (#MK115) assay to that of the former kit. The former kit employed O-phenylenediamine (OPD) as the substrate, and the precoated one employs 3, 3’, 5, 5’-tetramethylbenzidine (TMBZ) as the substrate. Assay was performed using 100-fold diluted serum samples. (n=20)
Storage and stability
This kit is shipped at 2 - 8°C and should be stored at 2 - 8°C if not used. Under this condition, the kit is stable until the expiration date on label.

References

Protocol summary
1. Prepare all reagents as directed in this Package Insert.
2. Bring all reagents to room temperature and prepare the solutions.
3. Add 100 μl of Standard or sample to appropriate wells, and incubate for 1 hour at 37°C.
4. Remove sample solution and wash the wells 3 times with 400 μl of Washing Buffer.
5. Add 100 μl of antibody-POD conjugate solution into wells and incubate at 37°C for 1 hour.
6. Aspirate solution from wells. Wash 4 times with ca. 400 μl of Washing Buffer per well, aspirating thoroughly between washes.
7. Add 100 μl of Substrate Solution to each well. Incubate 15 minutes at room temperature.
8. Add 100 μl of Stop Solution to all wells. Mix gently.
9. Read at 450 nm as soon as possible.