Introduction

Collagens (types I, II, III, IV and V) are synthesized as precursor molecules called procollagens. These contain additional peptide sequences, usually called "propeptides", at both the amino-terminal and the carboxy-terminal ends. The function of these propeptides is to facilitate the winding of procollagen molecules into a triple-helical conformation within the endoplasmic reticulum. The propeptides are cleaved off from the collagen triple helix molecule during its secretion, after which, the triple helix collagens polymerize into extracellular collagen fibrils. Thus, the amount of the free propeptides reflects stoichiometrically the amount of collagen molecules synthesized (a relationship analogous to that between the carboxy-terminal peptide of proinsulin and the endogenously produced insulin). Quantitative detection of collagen synthesis was first reported by Taubman et al (1) who performed competitive radioimmunoassays for procollagen type I carboxy-terminal peptide (PIP) using polyclonal antibodies. Similar methods using PIP as reference have been used in studying the correlation of collagen levels with certain health disorders, ie. bone diseases (2), alcoholic liver diseases (3), liver cirrhosis (4) and scirrhous (Borrmann type IV) adenocarcinoma of the stomach (5,6).
Procollagen Type I C-Peptide EIA Kit

Intended use
The Procollagen Type I C-peptide EIA Kit is an *in vitro* enzyme immunoassay (EIA) kit for quantitative determination of human, bovine or canine PIP in plasma, serum, cultured cell extracts, cell culture supernatants, or other biological fluids. *This kit is for research use only. It is not for use in diagnostic or therapeutic procedures.*

Principle
The PIP EIA Kit is a solid phase EIA based on a sandwich method that utilizes two mouse monoclonal anti-PIP antibodies to detect PIP by one-step procedure. One of the monoclonal antibodies has been pre-coated onto a microtiter-plate and blocked against non-specific binding. Samples, standard, and peroxidase (POD)-labelled anti-PIP antibody are simultaneously added to the wells of plates, and then incubated. During the incubation, PIP is bound to anti-PIP (solid phase) on one side and tagged by POD-anti-PIP on the other. The reaction between POD and substrate (H₂O₂ and tetramethylbenzidine) results in colour development with intensities proportional to the amount of PIP present in samples and standards. The amount of PIP can be quantitated by measuring the absorbance using an EIA plate reader. Accurate sample concentrations of PIP can be determined by comparing their specific absorbances with those obtained for the standards plotted on a standard curve.

Reagents and materials
Each PIP 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 × 12 strips)
The plate contains murine monoclonal antibody to PIP and blocking material.
Store at 2 〜 8 °C.

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

Vial 3. Standard - 1 vial (640 ng × 1)
The vial contains lyophilized PIP Standard.
Vial 4. Sample Diluent - 2 vials (11 ml × 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 ℃.

Vial 5. Substrate Solution - 1 vial (12 ml × 1)
The vial contains hydrogen peroxide and tetramethylbenzidine in a buffered solution. Store at 2 ～ 8 ℃.

B. Materials required but not provided
1. Reagents
   - Washing Buffer: Phosphate-buffered Saline (PBS)
     (Dissolve 8.0 grams of NaCl, 0.2 grams of KCl, 2.9 grams of Na₂HPO₄·2H₂O and 0.2 grams of KH₂PO₄ in 1000 ml of distilled water.)
     PBS tablet (Cat.#T900) is useful to prepare washing buffer.
   - Stop solution: 1 N H₂SO₄

2. Materials
   - Precision pipettes with disposable tips: 20 and 100 μl micropipettes, 10 ~ 200 μl adjustable multiwell pipetter or 20 μl and 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 (photometric range: ~ 3 OD)
   - 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 surfaces.
  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

Venous blood samples are collected aseptically. Serum is suitable for use in the assay, however, plasma, cultured cell extracts or cell culture supernatant can be also used. Remove the serum or plasma 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. Samples may be stored up to 12 hours at 2 ~ 10 °C. If the length of time between sample collection and assay is to exceed 12 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. PBS containing 0.5 % Triton X ~ 100, 1mM EDTA and 1mM Phenylmethylsulfonyl fluoride (pH7.2) should be used for preparation of cell extracts.

It is necessary to dilute human blood sample refer to sample dilution curve in basal data. (probably 5 ~ 10 folds)

Preparation of solutions

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

Solution 1. Antibody - POD Conjugate Solution
Dissolve the contents of Vial 2 in 1 ml 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 distilled water. Slowly roll for approximately 10 minutes or let stand and sporadically mix gently. The standard solution contains 640 ng PIP/ml. A dilution series can be formed by mixing the standard solution and Sample Diluent (Vial 4) for establishing the calibration curve, e.g.:

<table>
<thead>
<tr>
<th>Final conc. (ng/ml)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>40</th>
<th>80</th>
<th>160</th>
<th>320</th>
<th>640</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample Diluent</td>
<td>400 μl</td>
<td>393.75 μl</td>
<td>387.5 μl</td>
<td>375 μl</td>
<td>350 μl</td>
<td>300 μl</td>
<td>200 μl</td>
<td>-</td>
</tr>
<tr>
<td>Standard Solution</td>
<td>-</td>
<td>6.25 μl</td>
<td>12.5 μl</td>
<td>25 μl</td>
<td>50 μl</td>
<td>100 μl</td>
<td>200 μl</td>
<td>400 μl</td>
</tr>
</tbody>
</table>

Solution 3. Stop Solution (1N H2SO4)
Add 5.8 ml concentrated H2SO4 carefully to approximately 180 ml of distilled water (acid MUST be added to water, not vice versa). Add distilled water to a final volume of 200 ml. Mix well.
Store at 2 ~ 26 °C for up to 6 months.

Stability of solutions

Solution 1. The reconstituted lyophilisate is stable for 1 week stored at 4 °C, or for 1 month stored at — 20 °C. Do not repeat freeze-thaw cycle.
Solution 2. The reconstituted lyophilisate is stable for 1 week stored at 4 °C, or for 1 month stored at — 20 °C. Do not repeat freeze-thaw cycle.
**Procedure**

Duplicated 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.

1. **Immunological reaction**: Transfer 100 μ l of antibody-POD conjugate solution (Solution 1) into one well, and subsequently add 20 μ l sample or standard (Solution 2). Mix, seal the microtiter plate (e.g. with a foil) and stand for 3 hours at 37 °C. All sample and standard solution should be added within 5 minutes.

2. Remove contents by suction and wash the wells 4 times with ca.400 μ l of PBS; between the separate washing steps empty out the microtiter plate and vigorously tap onto paper towel, especially after the last washing.

3. **Substrate incubation**: Add 100 μ l of Substrate Solution (vial 5) into each well and incubate at room temperature (20 ～ 30 °C ) for 15 minutes.

4. Add 100 μ l of Stop Solution (Solution 3) into each well in same order as for substrate. Tap plate gently to mix.

5. 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**: At the first step of the Procedure on page 5, it is recommended to mix the antibody-POD conjugate solution and sample or standard by shaking. But, during the subsequent 3 hour incubation, the plate should not be shaken. Just leave it still. During the incubation at 37 °C or room temperature, the microtiter plate should be covered with an appropriate film to prevent the evaporation of the solution.

**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 PIP 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 PIP concentration (ng/ml) from the horizontal axis.
Performance characteristics

1. **Range of standard curve**: 10 ~ 640 ng/ml.

2. **Specificity**: This kit specifically measures PIP with no detectable cross reaction with human fibronectin, vitronectin, laminin, collagen type I, or collagen type III.
   This kit cannot be used to measure mouse PIP.

3. **Assay time**: Three and a half hour.

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, bovine, or canine serum or plasma; culture supernatants, cell extracts.

7. **Specimen volume required**: If each test sample is run in duplicate, approximately 50 μl (i.e., 20 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 PIP concentration from the curve.

9. **Notes**: According to the assay results using control serum or urine, it could be possible to determine the concentration of antigen present in a biological. However, the measurement may be potentially disturbed by the unknown organic factors in serum, plasma or urine 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 dilution solution included in the kit and assay them again in another run.

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Note:
The antibodies used in this kit cross react with procollagen type I C-peptides from bovine, horse and dog.
The measurement may be disturbed, when it is performed with samples that includes animal serum (ex. Fetal Bovine Serum (FBS) and Horse Serum). It is recommended to perform the measurement with the kit under the serum-free condition.
Basal data

1. **Typical standard curve**
   Detection limit is 10 ng/ml.
   
   Typical Standard Curve
   (Do Not Use To Calculate Unknowns)
   
   Curve Fit: 4-Parameter
   \[ y = \frac{(A - D)}{(1 + (x/C)^B)} + D \]
   \[ A = 0.0491 \quad B = 0.933 \quad C = 542. \quad D = 4.34 \]

   ![Graph of typical standard curve](image)

<table>
<thead>
<tr>
<th>PIP (ng/ml)</th>
<th>640.0</th>
<th>320.0</th>
<th>160.0</th>
<th>80.00</th>
<th>40.00</th>
<th>20.00</th>
<th>10.00</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_{450}) nm</td>
<td>2.359</td>
<td>1.685</td>
<td>1.084</td>
<td>0.658</td>
<td>0.408</td>
<td>0.236</td>
<td>0.149</td>
<td>0.047</td>
</tr>
</tbody>
</table>

2. **Intra-assay precision (n=16)**
   Assay was carried out with 16 replicates of 3 samples containing different concentrations of PIP.

<table>
<thead>
<tr>
<th></th>
<th>Ave. (ng/ml)</th>
<th>S.D. (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>484.8</td>
<td>35.64</td>
<td>7.4</td>
</tr>
<tr>
<td>Sample B</td>
<td>87.3</td>
<td>6.282</td>
<td>7.2</td>
</tr>
<tr>
<td>Sample C</td>
<td>31.7</td>
<td>1.411</td>
<td>4.5</td>
</tr>
</tbody>
</table>

**Inter-assay precision** (performance 3 times)
Assay to assay precision with one laboratory was evaluated in three independent experiments.

<table>
<thead>
<tr>
<th></th>
<th>Ave. (ng/ml)</th>
<th>S.D. (ng/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>466.1</td>
<td>20.25</td>
<td>4.3</td>
</tr>
<tr>
<td>Sample B</td>
<td>90.7</td>
<td>4.349</td>
<td>4.8</td>
</tr>
<tr>
<td>Sample C</td>
<td>29.6</td>
<td>1.873</td>
<td>6.3</td>
</tr>
</tbody>
</table>
3. Recovery test
The recovery of PIP was tested by adding two samples out of five different level in various matrices.

<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>390.8</td>
<td>0.0</td>
<td>217.3</td>
<td>195.4</td>
<td>111</td>
</tr>
<tr>
<td>390.8</td>
<td>390.8</td>
<td>410.0</td>
<td>390.8</td>
<td>105</td>
</tr>
<tr>
<td>390.8</td>
<td>219.8</td>
<td>328.7</td>
<td>305.3</td>
<td>108</td>
</tr>
<tr>
<td>390.8</td>
<td>110.8</td>
<td>272.8</td>
<td>250.8</td>
<td>109</td>
</tr>
<tr>
<td>390.8</td>
<td>55.9</td>
<td>245.7</td>
<td>223.3</td>
<td>110</td>
</tr>
<tr>
<td>390.8</td>
<td>31.4</td>
<td>238.8</td>
<td>211.1</td>
<td>113</td>
</tr>
<tr>
<td>219.8</td>
<td>0.0</td>
<td>118.1</td>
<td>109.9</td>
<td>107</td>
</tr>
<tr>
<td>219.8</td>
<td>219.8</td>
<td>272.5</td>
<td>219.8</td>
<td>124</td>
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<tr>
<td>219.8</td>
<td>110.8</td>
<td>179.6</td>
<td>165.3</td>
<td>109</td>
</tr>
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<td>219.8</td>
<td>55.9</td>
<td>145.0</td>
<td>137.8</td>
<td>105</td>
</tr>
<tr>
<td>219.8</td>
<td>31.4</td>
<td>126.2</td>
<td>125.6</td>
<td>100</td>
</tr>
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<td>110.8</td>
<td>0.0</td>
<td>57.5</td>
<td>55.4</td>
<td>104</td>
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<td>110.8</td>
<td>110.8</td>
<td>111.6</td>
<td>110.8</td>
<td>101</td>
</tr>
<tr>
<td>110.8</td>
<td>55.9</td>
<td>99.5</td>
<td>83.3</td>
<td>119</td>
</tr>
<tr>
<td>110.8</td>
<td>31.4</td>
<td>83.7</td>
<td>71.1</td>
<td>118</td>
</tr>
<tr>
<td>55.9</td>
<td>0.0</td>
<td>25.6</td>
<td>27.9</td>
<td>92</td>
</tr>
<tr>
<td>55.9</td>
<td>55.9</td>
<td>56.6</td>
<td>55.9</td>
<td>101</td>
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<td>55.9</td>
<td>31.4</td>
<td>41.4</td>
<td>43.6</td>
<td>95</td>
</tr>
<tr>
<td>31.4</td>
<td>0.0</td>
<td>14.3</td>
<td>15.7</td>
<td>91</td>
</tr>
<tr>
<td>31.4</td>
<td>31.4</td>
<td>30.4</td>
<td>31.4</td>
<td>97</td>
</tr>
</tbody>
</table>

(unit: ng/ml)
4. Effect of anticoagulants

Effect of anticoagulants was examined with healthy samples by comparing the dilution curve of the samples which were simultaneously treated with different anticoagulants. It is necessary to dilute human blood sample appropriately.

\[
\begin{align*}
\text{Citrate plasma} & : y = 562.087x + 10.361, \quad r = 0.998 \\
\text{Heparinized plasma} & : y = 737.475x - 1.196, \quad r = 1.000 \\
\text{EDTA plasma} & : y = 715.951x + 8.274, \quad r = 0.998 \\
\text{Serum} & : y = 692.856x + 4.487, \quad r = 1.000
\end{align*}
\]
5. Influence of coexistence
   
The volume ratio of sample to co-existing substance is 4:1. Co-existing substance is shown in its final concentration.
6. Correlation with 384 wells format kit (Cat.#MK110)

Correlation with this kit (Cat.#MK101) between 384 wells format kit (Cat.#MK110) was examined to measure PIP amount of various concentrations of human serum.

The data showed good correlation between 96 wells format kit and 384 wells format kit. 384 wells format kit is useful for high-throughput assay.

* : Procollagen Type I C-Peptide EIA Kit Ver. PIP 384 wells format (Cat.#MK110) is custom made.

![Graph showing correlation between 96 wells and 384 wells format kits](image)

\[ y = 1.0234x + 0.106 \]

\[ R = 0.9871 \]

High sensitive assay with two-step procedure

This kit can be also used with two-step procedure. As the two-step procedure allows high sensitive result, this procedure is useful when sample may contain quite a small amount of antigen, or when a sample may contain the substance which inhibits Anti-body - POD conjugate, such as sodium azide.

Procedure :

1. Dilute Standard (640 ng) (Vial 3) to the concentration of 160 ng/ml. Setting 160 ng/ml as the highest concentration, prepare stepwise dilution series of standard solution.
2. Add each 100 μl of the prepared standard solution and sample into appropriate wells of a microtiter plate and incubate at 37 °C for 2 hours. A sample and standard solution should be added within 5 minutes.
3. Aspirate or decant the contents from wells. Wash 3 times with 400 μl of PBS per wells.
4. Add 100 μl of Solution 1 * to each well. Incubate at 37 °C for 1 hour.
5. Aspirate or decant the contents from wells. Wash 4 times with 400 μl of PBS per wells.
6. Add 100 μl of Substrate Solution (TMBZ) (Vial 5) into each well and incubate 15 minutes at room temperature (20 ~ 30 °C).
7. Add 100 μl of Stop Solution into each well in the same order that Substrate Solution (TMBZ) (Vial 5) was added, and mix well.
8. Read at 450 nm after adjusting 0 with distilled water. Color development remains stable for 1 hour after stopping reaction.

* : Please refer to the "Preparation of solutions" (page 4) for preparing Solution 1 and 2.
Application 1: Monitoring of PIP during differentiation into osteoblast

The amount of human PIP in culture supernatant of human Mesenchymal Stem Cell (hMSC; Lonza Cat. PT-2501) was monitored during differentiation into osteoblast. The supernatant was 3^6 - fold and 3^7 - fold diluted by the sample diluent as a result of the preliminary experiment.

As the bovine fetal serum that contains bovine PIP is in basal medium, it is necessary to subtract basal medium concentration (B) from sample concentration (A).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Concentration</th>
<th>Unit ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample (A)</td>
<td>Medium (B)</td>
</tr>
<tr>
<td>hMSC 3 day Control 3^6</td>
<td>× 3^6</td>
<td>16.67</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>× 3^7</td>
<td>4.73</td>
<td>0.00</td>
</tr>
<tr>
<td>hMSC 3 day Induction 3^6</td>
<td>× 3^6</td>
<td>19.24</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>× 3^7</td>
<td>8.56</td>
<td>0.00</td>
</tr>
<tr>
<td>Medium change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hMSC 7 day Induction 3^6</td>
<td>× 3^6</td>
<td>82.18</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>× 3^7</td>
<td>35.57</td>
<td>0.00</td>
</tr>
<tr>
<td>hMSC 10 day Induction 3^6</td>
<td>× 3^6</td>
<td>63.67</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>× 3^7</td>
<td>31.74</td>
<td>0.00</td>
</tr>
<tr>
<td>Medium change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hMSC 14 day Induction 3^6</td>
<td>× 3^6</td>
<td>111.50</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>× 3^7</td>
<td>54.51</td>
<td>0.00</td>
</tr>
<tr>
<td>Medium change</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>hMSC 21 day Induction 3^6</td>
<td>× 3^6</td>
<td>161.10</td>
<td>2.72</td>
</tr>
<tr>
<td></td>
<td>× 3^7</td>
<td>74.44</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<Result>

During the differentiation into osteoblast, the production of human PIP increased dramatically in culture supernatant.
### Application 2: Monitoring of PIP in culture supernatant in human osteoblast

Human Osteoblast Cells (NHOst Lonza Cat.#CC-2538) were cultured in the growth medium containing 10% FCS. The amount of human PIP in culture supernatant was monitored. The supernatant was 20-fold or 40-fold diluted by the sample diluent.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Concentration</th>
<th>(A) - (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHOst day 10</td>
<td>× 20</td>
<td>111.50</td>
<td>69.84</td>
</tr>
<tr>
<td></td>
<td>× 40</td>
<td>74.88</td>
<td>54.12</td>
</tr>
<tr>
<td>NHOst day 14</td>
<td>× 20</td>
<td>181.80</td>
<td>140.14</td>
</tr>
<tr>
<td></td>
<td>× 40</td>
<td>108.10</td>
<td>87.34</td>
</tr>
<tr>
<td>NHOst day 35</td>
<td>× 20</td>
<td>393.40</td>
<td>351.74</td>
</tr>
<tr>
<td></td>
<td>× 40</td>
<td>201.60</td>
<td>180.84</td>
</tr>
</tbody>
</table>

**< Result >**

High concentration of human PIP is detected in NHOst culture supernatant by the growth of NHOst.

Medium concentration (B) means bovine PIP that is contained in FCS.
Application 3: Measurement of bovine PIP in culture medium containing FCS (Fetal Calf Serum)

There are some differences of bovine PIP concentration between lots of FCS. The amount of PIP were measured in several medium containing 10% FCS. The supernatant was 20-fold or 40-fold diluted by the sample diluent.

PIP concentration in 10% FCS / RPMI1640 medium

<table>
<thead>
<tr>
<th>FCS Lot</th>
<th>Dilution</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. A company</td>
<td>× 3³</td>
<td>69.61</td>
</tr>
<tr>
<td></td>
<td>× 3⁴</td>
<td>21.14</td>
</tr>
<tr>
<td>2. B company</td>
<td>× 3³</td>
<td>88.04</td>
</tr>
<tr>
<td></td>
<td>× 3⁴</td>
<td>29.67</td>
</tr>
<tr>
<td>3. C company</td>
<td>× 3³</td>
<td>73.39</td>
</tr>
<tr>
<td></td>
<td>× 3⁴</td>
<td>29.04</td>
</tr>
<tr>
<td>4. D company</td>
<td>× 3³</td>
<td>109.40</td>
</tr>
<tr>
<td></td>
<td>× 3⁴</td>
<td>50.50</td>
</tr>
</tbody>
</table>

PIP concentration in Culture Supernatant of one cell line

<table>
<thead>
<tr>
<th>Sample</th>
<th>Dilution</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium only (B)</td>
<td>× 3³</td>
<td>70.93</td>
</tr>
<tr>
<td>(10% FCS)</td>
<td>× 3⁴</td>
<td>25.49</td>
</tr>
<tr>
<td>MES.SA (A) Human uterus sarcoma</td>
<td>× 3³</td>
<td>143.60</td>
</tr>
<tr>
<td></td>
<td>× 3⁴</td>
<td>73.92</td>
</tr>
</tbody>
</table>

< Result >

Human PIP concentration can be detected in 10% FCS/RPMI 1640 medium by dilution the cell culture supernatant × 3³ or × 3⁴ fold to subtract the amount of medium (B) from total value (A).
Procollagen Type I C-Peptide EIA Kit

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. Transfer 100 μl of antibody - POD conjugate solution into appropriate wells.
2. Add 20 μl of Standard or sample to the wells within 5 minutes, and incubate 3 hours at 37 °C.
3. Remove sample solution and wash the wells 4 times with 400 μl of PBS.
4. Add 100 μl of Substrate Solution to each well. Incubate at room temperature for 15 minutes.
5. Add 100 μl of Stop Solution to all wells. Mix gently.
6. Read at 450 nm as soon as possible.

Note: This product is intended to be used for research purpose only. They are not to be used for drug or diagnostic purposes, nor are they intended for human use. They shall not to be used products as food, cosmetics, or utensils, etc. Takara products may not be resold or transfered, modified for resale or transfer, or used to manufacture commercial products without written approval from TAKARA BIO INC. If you require licenses for other use, please call at +81 77 543 7247 or contact from our website at www.takara-bio.com.