**ab210966 – Human Pro-Collagen I alpha 1 SimpleStep ELISA® Kit**

For overview, typical data and additional information please visit [www.abcam.com/ab210966](http://www.abcam.com/ab210966)

**Storage and Stability:** Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent and Standard Preparation sections

### Materials Supplied

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
<th>Storage Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X Human Pro-Collagen I alpha 1 Capture Antibody</td>
<td>600 µL</td>
<td>4°C</td>
</tr>
<tr>
<td>10X Human Pro-Collagen I alpha 1 Detector Antibody</td>
<td>600 µL</td>
<td>4°C</td>
</tr>
<tr>
<td>Human Pro-Collagen I alpha 1 Lyophilized Recombinant Protein</td>
<td>2 Vials</td>
<td>4°C</td>
</tr>
<tr>
<td>Antibody Diluent CPI</td>
<td>6 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>10X Wash Buffer PT</td>
<td>20 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>5X Cell Extraction Buffer PTR</td>
<td>10 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>50X Cell Extraction Enhancer Solution Mix</td>
<td>1 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>TMB Development Solution</td>
<td>12 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>12 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Sample Diluent NS</td>
<td>50 mL</td>
<td>4°C</td>
</tr>
<tr>
<td>Pre-Coated 96 Well Microplate (12 x 8 well strips)</td>
<td>96 wells</td>
<td>4°C</td>
</tr>
<tr>
<td>Plate Seal</td>
<td>1</td>
<td>4°C</td>
</tr>
</tbody>
</table>

### Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 or 600 nm.
- Method for determining protein concentration (BCA assay recommended).
- Deionized water.
- PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.4).
- Multi- and single-channel pipettes.
- Tubes for standard dilution.
- Plate shaker for all incubation steps.
- Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

### Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips): adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations

**1X Cell Extraction Buffer PTR (For cell and tissue extracts only)** Prepare 1X Cell Extraction Buffer PTR by diluting 5X Cell Extraction Buffer PTR and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL 5X Cell Extraction Buffer PTR and 200 µL 50X Cell Extraction Enhancer Solution Mix thoroughly and gently. If required protease inhibitors can be added. If the lysate is viscous and difficult to pipette due to solubilized genomic DNA: Prepare 1X Cell Extraction Buffer PTR (without enhancer). Add enhancer to lysate after extraction.

For cell and tissue extract samples follow these instructions:

1. Reconstitute the human Pro-Collagen I alpha 1 standard sample by adding 1 mL Sample Diluent NS. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 31.250 pg/mL Stock Standard Solution.
2. Label eight tubes, Standards 1–8.
3. Add 468 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

![Graph showing dilution series](image)

**For serum, plasma, and cell culture supernatant samples follow these instructions:**

1. Reconstitute the human Pro-Collagen I alpha 1 standard sample by adding 1 mL 1X Cell Extraction Buffer PTR. Mix thoroughly and gently. Hold at room temperature for 10 minutes and mix gently. This is the 31,250 pg/mL Stock Standard Solution.
2. Label eight tubes, Standards 1–8.
3. Add 460 µL Sample Diluent NS into tube number 1 and 150 µL of Sample Diluent NS into numbers 2-8.
4. Use the Stock Standard to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:
**Preparation of extracts from adherent cells by direct lysis (alternative protocol)**

Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

**Plate Preparation**

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, reseal and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control. For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates). Differences in well absorbance or “edge effects” have not been observed with this assay.

**Assay Procedure**

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls and samples in duplicate.

1) Prepare all reagents, working standards, and samples as directed in the previous sections.

2) Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.

3) Add 50 µL of all sample or standard to appropriate wells.

4) Add 50 µL of the Antibody Cocktail to each well.

5) Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.

6) Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and blot it against clean paper towels to remove excess liquid.

7) Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm. Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes. Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

8) Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

9) Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

<table>
<thead>
<tr>
<th>Mode</th>
<th>Kinetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength</td>
<td>600 nm</td>
</tr>
<tr>
<td>Time</td>
<td>up to 15 min</td>
</tr>
<tr>
<td>Interval</td>
<td>20 sec - 1 min</td>
</tr>
<tr>
<td>Shake</td>
<td>Shake between readings</td>
</tr>
</tbody>
</table>

Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips: [www.abcam.com/protocols/the-complete-elisa-guide](http://www.abcam.com/protocols/the-complete-elisa-guide)

For technical support contact information, visit: [www.abcam.com/contactus](http://www.abcam.com/contactus)

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