ab325360 – Human Pro-Collagen I alpha 1 SimpleStep ELISA® Kit, Chemiluminescent

For the quantitative measurement of Pro-Collagen I alpha 1 in human serum, plasma (heparin), plasma (EDTA), plasma (citrate), cell culture supernatant, saliva, urine, and cell extract.
For research use only - not intended for diagnostic use.

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 Standard Preparation and Reagent preparation sections.

Limitations: All data, except standard curve, sensitivity, and saliva and urine validation data were collected using the colorimetric version of this kit (ab210966).

Materials Supplied

Item	Quantity 1 x 96 well	Quantity 10 x 96 well	Storage Condition
Human Pro-Collagen I alpha 1 Capture Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Pro-Collagen I alpha 1 Detector Antibody 10X	600 µL	10 x 600 µL	+4°C
Human Pro-Collagen I alpha 1 Lyophilized Recombinant Protein	2 Vials	10 x 2 Vials	+4°C
Antibody Diluent CPI2	6 mL	10 x 6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	2 x 50 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	10 x 1 mL	+4°C
Sample Diluent NS	50 mL	2 x 250 mL	+4°C
Wash Buffer PT 10X	20 mL	200 mL	+4°C
ChemiHRP Reagent A	3 mL	50 mL	+4°C
ChemiHRP Reagent B	3 mL	50 mL	+4°C
SimpleStep Pre-Coated Black 96-Well Microplate	96 Wells	10 x 96 Wells	+4°C
Plate Seal	1	10	+4°C

Materials Required, Not Supplied

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

Luminometer with the following settings: 0.5-1 second/well read time; summation mode (all wavelengths)
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for standard dilution.
Plate shaker for all incubation steps.
Optional: 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.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X 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 Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. 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.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CPI2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CPI2. Mix thoroughly and gently.

Lumi HRP Development Solution: Just prior to use, prepare Lumi HRP Development Solution by mixing equal volume of the ChemiHRP Reagent A and the ChemiHRP Reagent B. To make 3 mL of the Lumi HRP Development Solution combine 1.5 mL of ChemiHRP Reagent A and 1.5 mL of ChemiHRP Reagent B. Mix thoroughly and gently by inversion or slow pipetting (Avoid shaking or vortexing). Protect the prepared solution from light until use.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. **IMPORTANT:** If the protein standard vial has a volume identified on the label, reconstitute the Pro-Collagen I alpha standard by adding that volume of Diluent indicated on the label. Alternatively, if the vial has a mass identified, reconstitute the Pro-Collagen I alpha standard by adding 1 mL Diluent. Hold at room temperature for 10 minutes and mix gently. This is the 31,250 pg/mL **Stock Standard** Solution.
2. For **serum**, **plasma, cell culture supernatant, urine, and saliva samples measurements**, use Sample Diluent NS. For **cell extract samples measurements**, use 1X Extraction Buffer PTR.
3. Label nine tubes, Standards 1– 9.
4. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 204 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-9.
5. Use the **Stock Standard** to prepare the following dilution series. Standard #9 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	**Stock Standard**	96	204	31,250	10,000
2	Standard#1	75	150	10,000	3,333.33
3	Standard#2	75	150	3,333.33	1,111.11
4	Standard#3	75	150	1,111.11	370.37
5	Standard#4	75	150	370.37	123.46
6	Standard#5	75	150	123.46	41.15
7	Standard#6	75	150	41.15	13.72
8	Standard#7	75	150	13.72	4.57
9	Blank Control	0	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	**Range**
Serum	1:1,600 - 1:100
Plasma – Citrate	1:1,600 - 1:100
Plasma – EDTA	1:1,600 - 1:100
Plasma – Heparin	1:1,600 - 1:100
Cell Culture Media	≤ 25%
Urine	3.13 - 50%
Saliva	0.63 - 10%
IMR-90 Cell Extract	0.125 - 2 µg/mL

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:100 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate, EDTA, or heparin. Centrifuge samples at 2,000 x g for 10 minutes. Dilute samples at least 1:100 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:4 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Urine Centrifuge urine at 2,000 x g for 10 minutes to remove debris. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted urine samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Saliva Centrifuge saliva at 800 x g for 10 minutes to remove debris. Dilute samples at least 1:10 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from cell pellets Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 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.

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, resealed 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 30 minutes at room temperature on a plate shaker set to 750 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. Wash Buffer PT should remain in wells for at least 30 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 50 µL of prepared Lumi HRP Development Solution to each well and incubate for 1 minute in the dark on a plate shaker set to 750 rpm. Further optimization of incubation time vs signal strength can be performed if needed. Avoid introducing bubbles into the wells.
8. Measure the produced light of each well using a microplate luminometer with the following settings: 0.5-1 second/well read time in summation mode (all wavelengths). Relative light unit (RLU) readings may vary between luminometer models. It is recommended to configure instrument settings according to the manufacturer's specifications. Note: Relative light unit (RLU) values may change over the course of the 15-minute reading window.
9. Analyse the data as described below.

Mode:	Luminescence
Instrument settings:	Endpoint
Detection Mode:	All wavelengths
Read Time:	0.5-1 sec
Read:	Top

Note For microplate readers with Pre-Read Optimization option, the Read Height as well as Microplate Optimization is recommended before the first read.

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

Technical Support

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of human Pro-Collagen I alpha 1. The antibodies in the kit were designed against the N-terminal pro-peptide.

Native signal was detected in serum, plasma (heparin), plasma (EDTA), plasma (citrate), saliva, urine, and cell extract sample types.

Spiked protein experiments were used to validate cell culture supernatant sample types.

Milk, CSF, and tissue extract samples have not been tested with this kit.

SPECIES REACTIVITY

Other species reactivity was determined by measuring 1:100 serum sampled of various species, interpolating the protein concentrations from the human standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in human serum assayed at the same dilution.

Reactivity < 3% was determined for the following species: Mouse, Rat, Cow

Other species reactivity not determined.

CALCULATION

- Preconfigured protocols are available when using SoftMax Pro software from Molecular Devices.
- Calculate the average chemiluminescence value for the blank control (zero) standards. Subtract the average blank control standard chemiluminescence value from all other chemiluminescence values.
- Create a standard curve by plotting the average blank control subtracted chemiluminescence value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
- Note: Most chemiluminescence reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g. linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted chemiluminescence values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating chemiluminescence values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at chemiluminescence values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	1,608	1,608	1,608
4.57	2,540	2,458	2,499
13.72	5,395	4,779	5,087
41.15	14,953	14,592	14,772.5
123.46	47,428	45,775	46,601.5
370.37	145,270	144,180	144,725
1,111.11	447,040	441,430	444,235
3,333.33	1,398,400	1,308,500	1,353,450
10,000	3,894,100	3,942,400	3,918,250

Table 1. Example of human Pro-Collagen I alpha 1 standard curve in Sample Diluent NS. The Pro-Collagen I alpha 1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	RLU		Mean RLU
	1	2	
0	1,860	1,860	1,860
4.57	3,090	3,010	3,050
13.72	5,688	5,635	5,662
41.15	12,540	13,064	12,802
123.46	36,064	35,881	35,973
370.37	107,500	118,570	113,035
1,111.11	327,690	362,800	345,245
3,333.33	1,039,200	1,061,100	1,050,150
10,000	3,129,800	3,176,100	3,152,950

Table 2. Example of human Pro-Collagen I alpha 1 standard curve in 1X Cell Extraction Buffer PTR. The Pro-Collagen I alpha 1 standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	8	3.2 pg/mL
1X Cell Extraction Buffer PTR	16	2.2 pg/mL

Recovery

Three concentrations of Pro-Collagen I alpha 1 were spiked in duplicate to the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
1:200 Human Serum	93	91 - 94
1:200 Human Plasma – Citrate	106	102 - 110
1:200 Human Plasma – EDTA	108	105 - 114
1:200 Human Plasma – Heparin	101	94 - 107
25% Human Urine	100	95 - 108
5% Human Saliva	93	90 - 95
25% Cell Culture Media*	99	97 - 101

*Media is RPMI 1640 without 10% fetal calf serum.

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Pro-Collagen I alpha 1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	1:100 Human Serum	1:100 Human Plasma (Citrate)	1:100 Human Plasma (EDTA)	1:100 Human Plasma (Heparin)	50% Human Urine	10% Human Saliva
Undiluted	pg/mL	1,635.4	1,538.2	1225.6	1,125.3	2,164.5	553.6
	% Expected value	100	100	100	100	100	100
2	pg/mL	713.1	679.0	555.7	514.1	1,075.1	282.8
	% Expected value	87	88	91	91	99	104
4	pg/mL	329.6	316.7	264.4	237.7	542.5	137.1
	% Expected value	81	82	86	84	100	101
8	pg/mL	167.1	162.1	138.3	124.6	246.0	68.9
	% Expected value	82	84	90	89	91	101
16	pg/mL	86.9	83.4	68.9	62.6	108.7	38.3
	% Expected value	85	87	90	89	80	113

Recombinant Pro-Collagen I alpha 1 was spiked into the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	25% Cell Culture Media
Undiluted	pg/mL	1,043.8
	% Expected value	100
2	pg/mL	522.4
	% Expected value	100
4	pg/mL	259.1
	% Expected value	99
8	pg/mL	129.6
	% Expected value	99
16	pg/mL	65.8
	% Expected value	101

Native Pro-Collagen I alpha 1 was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	2 µg/mL IMR-90 Extract
Undiluted	pg/mL	1,612.4
	% Expected value	100
2	pg/mL	808.7
	% Expected value	100
4	pg/mL	402.2
	% Expected value	100
8	pg/mL	205.0
	% Expected value	102
16	pg/mL	101.7
	% Expected value	101

Precision

Mean coefficient of variations of interpolated values of Pro-Collagen I alpha 1 from three concentrations of human serum within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	1.8	3.0

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

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