

**STRATUM Medical**

6199 Cornerstone Court East #111  
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 www.StratumMedical.com

**CERTIFICATE OF ANALYSIS***Protein Content and Composition by Amino Acid Analysis***Sample Preparation**

Dilutions of collagen solutions were prepared by dispensing 250 µL aliquots of collagen into glass vials (the sample weight is recorded) and adding 2.35 mL of 20 mM hydrochloric acid (the total diluted sample weight is recorded). Collagen sample aliquots of 1,000 µL were dispensed into vials and the weight recorded. The vials were placed in a vacuum desiccator and 1 mL of concentrated hydrochloric acid was added to each sample, and the desiccator was evacuated, and heated at 110 – 120 °C for 16 hours. After acid hydrolysis, the samples were cooled to room temperature, opened, and evaporated to dryness. Samples were reconstituted with 1,000 µL of 10 mM hydrochloric acid. Using the Waters AccQ•Tag amino acid analysis method, samples and amino acid standards were derivatized according to the manufacturer's conditions (10 µL sample plus 70 µL borate buffer plus 20 µL AccQ•Fluor Reagent) and analyzed by HPLC using the manufacturer's column and buffer system with fluorescent detection.

**Amino Acid Composition**

Amino acid composition was determined by dividing the peak area of each amino acid by the corresponding peak area of the amino acid standard injected at 100 pmoles per injection to determine pmoles per injection. The pmoles of each amino acid were summed and the total pmoles of all amino acids divided by 1,000 that was used to divide into the individual amino acid to express each amino acid in residues per 1,000 total residues.

<u>Amino Acid</u>	<u>Literature Composition</u>	<u>Average PureCol Lot 2370-61</u>	<u>Average PureCol Lot 7503</u>	<u>Average PureCol Lot 7626</u>	<u>Average P/N 5198 Lot 7647</u>
Hyp	104	94.6	96.0	96.4	89.0
Asp	43	45.2	45.4	45.2	45.0
Ser	33	32.8	32.6	21.7	32.8
Glu	71	69.3	69.7	69.9	71.2
Gly	335	338.7	338.6	342.5	329.5
His	6	7.5	7.3	8.2	7.5
Arg	50	51.9	51.8	52.5	51.1
Thr	17	17.2	17.1	17.5	25.1
Ala	110	106.0	106.1	107.5	106.4
Pro	120	129.7	128.6	130.6	128.7
Cys	0	0.0	0.0	0.0	0.0
Tyr	2	1.5	1.5	1.4	2.3
Val	26	21.7	21.5	22.0	21.3
Met	6	9.3	9.3	9.3	13.7
Lys	33	26.5	26.3	26.6	26.2
Ile	9	13.1	13.1	13.2	12.7
Leu	23	23.2	23.2	23.5	24.7
Phe	12	11.7	11.8	12.0	12.6

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**CERTIFICATE OF ANALYSIS****Protein Content**

Protein content was determined by multiplying the individual pmoles of each amino acid by its molecular weight and summing the individual amino acid weight contribution and then by the dilutions used. Protein content is expressed in mg/mL.

	PureCol <u>Lot 2370-61</u>	PureCol <u>Lot 7503</u>	PureCol <u>Lot 7626</u>	P/N 5198 <u>Lot 7647</u>
Dilution	3.30	3.09	2.94	3.88
Neat #1	3.27	3.16	3.09	3.78
Neat #2	3.20	3.17	3.12	3.84
Average	3.26	3.14	3.05	3.83