



## RayBiotech, Inc.

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# Certificate of Analysis and Data Sheet Recombinant Human Like Collagen

**Catalog No.**  
228-10264

**Source**  
*Escherichia Coli.*

### ***Introduction***

Collagen remained the initial functions of collagen and had some new characters because of its structure, such as reversible colloid with heat and easy processed without molecular reduction and so on. However, animal collagen extracted from animal tissues was insoluble except for the molecular weight reduction.

The fetal shortcomings of animal collagen restricted the application is its harmful virus, but Human-like Collagen made up these drawbacks. Collagen (extracted from animal skin, tendons and hides) is potential risk in virus with the potential to cause side effect and the potential for the contamination of bovine collagen with the fatal mad cow disease. Collagen dissolves in water, which is another advantage to compete with animal collagen. It is different from hydrolyzed collagen produced by hydrolyzing animal collagen to short peptide linkage with a lower molecular weight.

Human-like collagen was expressed by human collagen gene in host, so immunogenicity could be lessened much by compared with animal collagen after it entered into human body.

### ***Description***

Collagen Human Recombinant produced in E.Coli is a non-glycosylated polypeptide chain a molecular mass of 96,000 Dalton containing human collagen's cDNA transcribed reversely from mRNA. The Collagen is purified by proprietary chromatographic techniques.

### ***Physical Appearance***

Sterile Filtered White lyophilized (freeze-dried) powder.

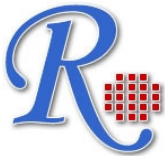
### ***Formulation***

The protein was lyophilized from a sterile solution containing no additives.

### ***Solubility***

It is recommended to reconstitute the lyophilized Collagen in sterile 18MΩ-cm H<sub>2</sub>O not less than 100μg/ml, which can then be further diluted to other aqueous solutions.

**The products are furnished for LABORATORY RESEARCH USE ONLY.  
Not for diagnostic or therapeutic use.**



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### ***Stability***

Lyophilized Collagen although stable at room temperature for 3 weeks, should be stored desiccated below -18°C. Upon reconstitution Collagen should be stored at 4°C between 2-7 days and for future use below -18°C.

For long term storage it is recommended to add a carrier protein (0.1% HSA or BSA).  
Please prevent freeze-thaw cycles.

### ***Purity***

Greater than 95.0% as determined by (a) Analysis by RP-HPLC.  
(b) Analysis by SDS-PAGE.

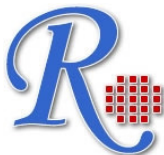
### ***Amino acid sequence***

The sequence of the first fifteen N-terminal amino acids was determined and was found to be MDPVVLQRRDWENPG. Amino acid %Asp 0.39; Thr 0.16; Ser 5.70; Pro 18.87; Gly 29.17; Ala 13.38; Val .71; Met .18; Ile 0.16; Leu 8.04; Tyr 0.90; Phe 0.15; Lys 0.27; His 0.15; Arg 0.03; Gln 17.40; Hydro Pro 4.34.

### ***Various uses of activity***

- A. Bio-Absorption
- B. Cell-Adhesion
- C. Mini-immunogenicity and neovascularization
- D. Promoting the formation of epithelial cell
- E. Biocompatibility
- F. Absorption water
- G. Against UV radiation.
- H. Repairing defected skin
- I. Removing pock, acne, spot
- J. Dispelling black eye sockets, eye dropping.

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### ***Biological Activity***

#### **1. Cultivation of BHK-21 and 2BS cell line**

BHK-21 or 2BS cells were plated onto the bottom of the 96-well cell culture plates at the density of  $1 \times 10^3$  cells/well, respectively, with 0.1ml RPMI1640 contained 10% fetal bovine serum in the well. BHK-21 or 2BS cells were incubated in standard conditions (at 37°C with 5% CO<sub>2</sub> in a humidified incubator) for 24 hours.

#### **2. Biochemical activity analysis of Human-like Collagen Complete culture media was removed after 24 hours, Collagen was added at various concentrations**

0.01%, 0.03%, 0.05%, 0.07%, 0.09%, 0.10%, 0.11%, 0.13% and a complete medium was used as blank control. Cells were incubated in standard conditions (at 37°C with 5% CO<sub>2</sub> in a humidified incubator). The morphology of cell was observed by reverse microscope after 24hr, 48hr, 72hr, 96hr, and 120hr. Thereafter, the viable cell count was determined by a colorimetric method using MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5 diphenyl tetrazolium bromide) after 24hr, 48hr, 72hr, 96hr and 120hr. A490 was evaluated by ELISA reader.

#### **3. Results**

The results demonstrate that Collagen has no significant effect on cell morphology. MTT analysis shows that Collagen has remarkable effect on cell proliferation when its concentration is higher than 0.03%. Cell proliferation was improved by 40% to 60% compared to control.

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