**DESCRIPTION**

**Source**  
Chinese Hamster Ovary cell line, CHO-derived human Collagen II protein  
Gln26-Arg266 & Gly957-Leu1487, with an N-terminal 5-His tag  
Accession # P02458

**N-terminal Sequence Analysis**  
His & Asp1242

**Predicted Molecular Mass**  
78 kDa

**SPECIFICATIONS**

**SDS-PAGE**  
92-100 kDa, 65-70 kDa, 36 kDa, reducing conditions

**Activity**  
Measured by the cleavage of its C-terminal propeptide by Recombinant Human BMP-1/PCP (Catalog # 1927-ZN).  
>50% of full-length Pro-Collagen II is cleaved by Recombinant Human BMP-1/PCP (Catalog # 1927-ZN), as measured under the described conditions.

**Endotoxin Level**  
<1.0 EU per 1 µg of the protein by the LAL method.

**Purity**  
>85%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation**  
Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

**Activity Assay Protocol**

**Materials**
- Dilution Buffer: 50 mM Tris, 5 mM CaCl₂, pH 7.5
- Assay Buffer: 50 mM Tris, 5 mM CaCl₂, 150 mM NaCl, pH 7.5 (TCN)
- Recombinant Human Pro-Collagen II (rhCOL-2A1) (Catalog # 7589-CL)
- Recombinant Human BMP-1/PCP (rhBMP-1) (Catalog # 1927-ZN)
- Reducing SDS-PAGE Sample Buffer
- SDS-PAGE or Western Blot

**Assay**
1. Dilute rhCOL-2A1 to 75 µg/mL in Dilution Buffer.
2. Dilute rhBMP-1 to 10 µg/mL in Assay Buffer.
3. Combine one volume of diluted rhCOL-2A1 with three volumes of diluted rhBMP-1. For controls, combine one volume of rhCOL-2A1 with three volumes of Assay Buffer, as well as three volumes of rhBMP-1 with one volume of Assay Buffer.
4. Incubate reaction vials and controls at 37 °C for one hour.
5. After incubation, combine rhCOL-2A1/rhBMP-1 reaction mixtures and controls with reducing SDS-PAGE gel buffer at a 2:1 (reaction mixture:gel buffer) ratio (v/v). Mix and incubate samples at 95-100 °C for 3-5 minutes to stop reactions.
6. Load 40 µL (0.5 µg of rCOL-2A1) per lane and analyze the cleavage by SDS-PAGE followed by protein staining and/or Western blot.
7. Activity calculation:
   \[
   \% \text{ Cleavage} = \left( \frac{1 - \% \text{ full-length rhCOL-2A1 (reaction)}}{\% \text{ full-length rhCOL-2A1 (control)}} \right) \times 100\%
   \]

**Final Assay Conditions**  
Per Lane:
- rhCOL-2A1: 0.5 µg
- rhBMP-1: 0.2 µg

**PREPARATION AND STORAGE**

**Shipping**
The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage**
Use a manual defrost freezer and avoid repeated freeze-thaw cycles.
- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

**BACKGROUND**

Type II collagen is an essential component of cartilage. It is essential for skeletal development and for the ability of cartilage to resist compressive forces. Type II collagen is a homotrimer of α1(II) chains. Defects in Type II collagen are the cause of numerous diseases, including chondrodysplasias and arthritis (1). This recombinant mini pro-α1(II) collagen consists of the N-terminal propeptide, 108 Gly-X-Y repeats, and the C-terminal propeptide. Proteolytic processing of the C-terminal propeptide by the procollagen C-proteinase (BMP-1) releases chondrocalcin, a calcium binding protein.

**References:**

---

**Rev. 4/27/2018 Page 1 of 1**