

DESCRIPTION

Source Mouse myeloma cell line, NS0-derived human Collagen III alpha 1/COL3A1 protein
Thr1097-Leu1466, with an N-terminal 6-His tag
Accession # NP_000081

N-terminal Sequence Analysis His

Predicted Molecular Mass 40 kDa

SPECIFICATIONS

SDS-PAGE 41-46 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 III α 1 is cleaved by recombinant human BMP-1, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 μ g of the protein by the LAL method.

Purity >75%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 μ g per lane.

Formulation Supplied as a 0.2 μ m filtered solution in Sodium Acetate and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 25 mM HEPES, 0.01% Brij, pH 7.5
 - Recombinant Human Pro Collagen III α 1/COL3A1 (rhPro-COL3A1) (Catalog # 7294-CL)
 - Recombinant Human BMP-1/PCP (rhBMP-1) (Catalog # 1927-ZN)
 - Reducing SDS-PAGE Sample Buffer
 - SDS-PAGE or Western Blot

- Assay**
1. Dilute rhPro-COL3A1 to 75 μ g/mL in Assay Buffer.
 2. Dilute rhBMP-1 to 5 μ g/mL in Assay Buffer.
 3. Combine one volume of diluted rhPro-COL3A1 with three volumes of diluted rhBMP-1. For controls, combine one volume of rhPro-COL3A1 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 rhPro-COL3A1/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 μ L (0.5 μ g of rhPro-COL3A1) per lane and analyze the cleavage by SDS-PAGE followed by protein staining and/or Western blot.
 7. Activity calculation:
$$\% \text{ Cleavage} = \left[1 - \frac{\% \text{ full-length rhPro-COL3A1 (reaction)}}{\% \text{ full-length rhPro-COL3A1 (control)}} \right] \times 100\%$$

- Final Assay Conditions** Per Lane:
- rhCOL3A1: 0.5 μ g
 - rhBMP-1: 0.1 μ g

PREPARATION AND STORAGE

Shipping The product is shipped with polar packs. 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, -20 to -70 °C as supplied.
 - 3 months, -20 to -70 °C under sterile conditions after opening.

BACKGROUND

Collagen type III is found in most soft connective tissues, such as skin, lung, and the vascular system, often in association with type I collagen. It is a trimer of α 1(III) chains that are linked by interchain disulfide bonds. Defective collagen type III is a cause of Ehlers-Danlos syndrome types III and IV and arterial aneurisms (1). This recombinant mini pro- α 1(III) collagen consists of a triple-helical region of Gly-Xaa-Yaa repeats, a short non-helical region, and the C-terminal propeptide. The C-terminal propeptide can be removed by the procollagen C-proteinase (BMP-1).

References:

1. Kuivaniemi H. *et al.* (1997) Hum. Mutat. 9:300.

PRODUCT SPECIFIC NOTICES

Coomassie is a registered trademark of Imperial Chemical Industries Ltd.