Human Procollagen Type I C-Peptide (PIP) (Cat.# M011)

Application: Western Blotting with Anti-Human Procollagen Type I C-peptide (PIP)

Procollagens are precursor molecules of collagens (types I, II, III, IV and V). Procollagens contain additional peptide sequences, called "propeptides", at both the N and C terminal ends that facilitate the winding of procollagen molecules into a triple-helical conformation within the endoplasmic reticulum. This application note describes the use of a monoclonal antibody raised against procollagen type 1C-peptide (PIP) (Cat.# M011) to investigate the extracellular matrix components secreted by cultured bovine cells.

Methods

Bovine periosteal cells were cultured in medium with and without ascorbic acid. Cell supernatants were collected after 3, 4, and 5 weeks of culture. Samples for analysis were prepared with the ProteoExtract Protein Precipitation Kit (Cat.# 539180-1KIT, Calbiochem) and dissolved in Laemmli sample buffer for SDS-PAGE. For western blots performed with the Anti-Human Procollagen Type I C-peptide (PIP), samples were prepared under non-reducing conditions and were not heat-denatured prior to electrophoresis. Samples (20 µg) were run on a 7.5% SDS polyacrylamide gel and blotted to a membrane according to standard laboratory procedures. The blots were incubated overnight with primary antibody (1:1000). After washing, the blots were incubated with Envision Polymer (DAKO) (1:50) as secondary antibody. Signal detection was accomplished by chemiluminescence.

Antibodies Used:

- Anti-Human Procollagen Type I C-peptide (PIP) (Clone PC5-5, Cat.# M011) (Capable of detecting both human and bovine antigens)
- Anti-bovine collagen type I (Abcam)

Results

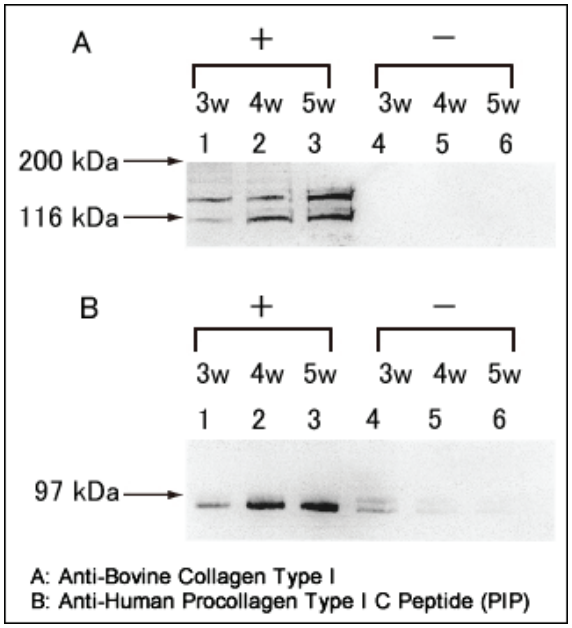

Figure 1. Western blot detection of collagen type I (A) and procollagen type I C-peptide (B). The supernatant of cultured bovine cells treated (+) or untreated (-) with ascorbic acid for 3, 4, or 5 weeks (3w, 4w, and 5w, respectively) were assayed. These data are reprinted from the referenced literature with the publisher's permission.

Conclusions

The target bands corresponding to procollagen type I C-peptide and collagen type I were detected in the culture supernatants of cells that were treated with ascorbic acid.

Reference

Akiyama, M. and Nakamura, M. (2009) Bone regeneration and neovascularization processes in a pellet culture system for periosteal cells. *Cell Transplantation,* **18**:443-452.