Human Pro Collagen I α1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG
Catalog Number: AF6220

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human Pro-Collagen I alpha 1 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human COL3A1 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human Collagen I Gln23-Lys277, Gly1094-Leu1464 Accession # P02452
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

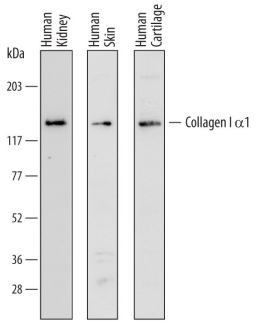
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Western Blot	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below
Immunoprecipitation	5 µg/mL	IMR-90 human lung fibroblast cell line
Simple Western	10 µg/mL	IMR-90 human lung fibroblast cell line

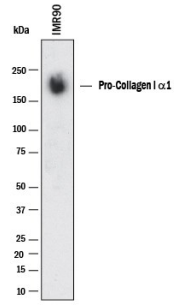
DATA

Western Blot



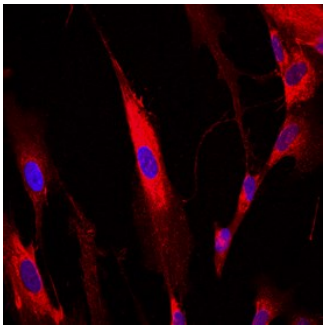
Detection of Human Pro-Collagen I alpha 1 by Western Blot. Western blot shows lysates of human kidney tissue, human skin tissue, and human cartilage tissue. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Pro-Collagen I alpha 1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6220) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # Catalog # HAF016). A specific band was detected for Pro-Collagen I alpha 1 at approximately 140 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Western Blot



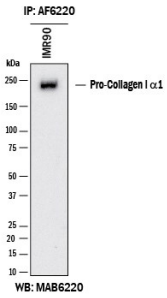
Detection of Human Collagen I by Western Blot. Western blot shows lysates of IMR-90 human lung fibroblast cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human Pro Collagen I α1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6220) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Collagen I at approximately 170 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Immunocytochemistry



Pro-Collagen I alpha 1 in IMR-90 Human Cell Line. Pro-Collagen I alpha 1 was detected in immersion fixed IMR-90 human lung fibroblast cell line using Sheep Anti-Human Pro-Collagen I alpha 1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6220) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # Catalog # NL010) and counterstained with DAPI (blue). View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Immunoprecipitation



Detection of Human N-Pro Collagen I by Immunoprecipitation. Human N-Pro Collagen I was immunoprecipitated from 500 µg of IMR-90 human lung fibroblast cell line lysates with 5 µg of Sheep Anti-Human N-Pro Collagen I Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6220). The N-Pro Collagen I-antibody complexes were absorbed using Protein A or Protein G. Immunoprecipitated human N-Pro Collagen I was detected by Western blot using 1 µg/mL of Mouse Anti-Human N-Pro Collagen I Monoclonal Antibody (Catalog # MAB6220) under reducing conditions and using Western Blot Buffer Group 1.

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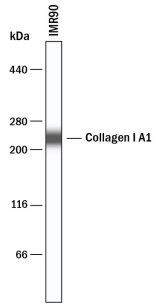
Simple Western



Detection of Human Collagen I α1 by Simple Western™. Simple Western lane view shows lysates of IMR-90 human lung fibroblast cell line, loaded at 0.2 mg/mL. A specific band was detected for Collagen I α1 at approximately 232 kDa (as indicated) using 10 µg/mL of Sheep Anti-Human Pro Collagen I α1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6220) followed by 1:50 dilution of HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

BACKGROUND

Type I collagen is the most abundant structural protein of connective tissues such as skin, bone and tendon. It is synthesized as a procollagen molecule which is characterized by a 300 nm triple helical domain flanked by globular N- and C-terminal propeptides (1). The triple helical domain contains Gly-Xaa-Yaa triplets where Xaa and Yaa are frequently proline and hydroxyproline, respectively. The non-helical propeptides are removed by procollagen N- and C-proteinase activities so that the mature triple helices can self-assemble into collagen fibrils that provide tensile strength to tissues (1). Type I collagen is a heterotrimer that consists of two α1(I) chains and one α2(I) chain, although homotrimers consisting of three identical α1(I) chains have also been described (2). This recombinant mini pro-α1(I) collagen consists of a shortened α1(I) chain with following domain structure from N- to C-terminus: N-propeptide, N-telopeptide, the 33 most N-terminal Gly-Xaa-Yaa repeats, the 33 most C-terminal Gly-Xaa-Yaa repeats, C-telopeptide and C-propeptide. The preparation contains a mixture of the full-length molecule, pN collagen I(α1) and the C-terminal propeptide. This truncated pro-α1(I) collagen is a substrate for procollagen N-proteinase and procollagen C-proteinase.

References:

1. Canty, E.G. *et al.* (2005) J. Cell Sci. **118**:1341.
2. Han, S. *et al.* (2008) J. Mol. Biol. **383**:122.

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