Mouse Collagen I α1 Antibody

Monoclonal Rat IgG_{2B} Clone # 1102924

Catalog Number: MAB11700

DESCRIPTION

Species Reactivity	Mouse
Specificity	Detects a synthetic peptide specific for mouse COL1A1 around amino acid 215 in Direct ELISA.
Source	Monoclonal Rat IgG_{2B} Clone # 1102924
Purification	Protein A or G purified from hybridoma culture supernatant
Immunogen	Synthetic Peptide Accession # P11087
Formulation	Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose.

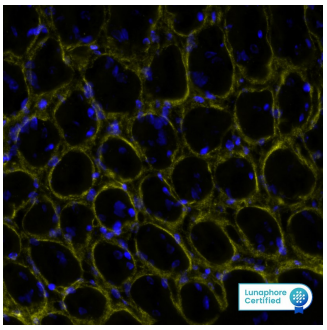
APPLICATIONS

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	MEF mouse embryonic feeder cells
Immunocytochemistry	3-25 µg/mL	Immersion fixed 3T3-L1 mouse embryonic fibroblast adipose-like cell line
Multiplex Immunofluorescence	25 µg/mL	Immersion fixed paraffin-embedded sections of mouse colon
Immunohistochemistry	3-25 µg/mL	Perfusion fixed paraffin-embedded sections of mouse kidney and pancreas
Simple Western	10 µg/mL	MEF mouse embryonic feeder cells

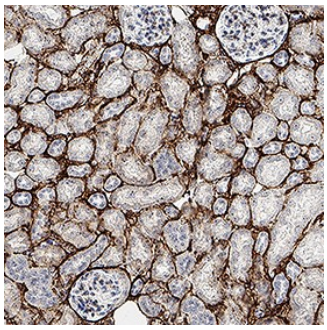
DATA

Multiplex Immunofluorescence



COL1A1 in Mouse Colon via seqIF™ staining on COMET™ COL1A1 was detected in immersion fixed paraffin-embedded sections of mouse Colon using Rat Anti-Mouse COL1A1, Monoclonal Antibody (Catalog #MAB11700) at 25ug/mL at 37° Celsius for 4 minutes. Before incubation with the primary antibody, tissue underwent an all-in-one dewaxing and antigen retrieval preprocessing using PreTreatment Module (PT Module) and Dewax and HIER Buffer H (pH 9; Epredia Catalog # TA-999-DHBH). Tissue was stained using the Alexa Fluor™ 647 Goat anti-Rat IgG Secondary Antibody at 1:200 at 37 ° Celsius for 2 minutes. (Yellow; Lunaphore Catalog # DR647RT) and counterstained with DAPI (blue; Lunaphore Catalog # DR100).. Specific staining was localized to connective tissue. Protocol available in COMET™ Panel Builder.

Immunohistochemistry



Detection of Collagen I α1 in Mouse Kidney. Collagen I α1 was detected in perfusion fixed paraffin-embedded sections of mouse kidney using Rat Anti-Mouse Collagen I α1 Monoclonal Antibody (Catalog # MAB11700) at 5 µg/ml overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using the HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

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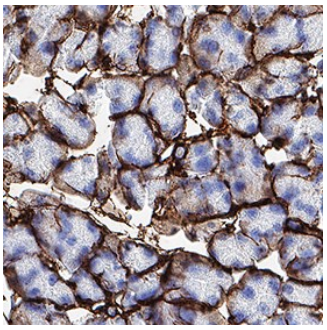
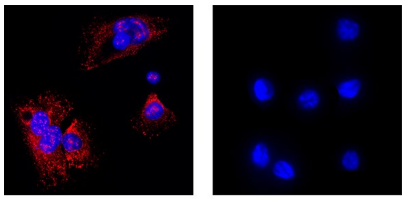
Immunohistochemistry



Detection of Collagen I α1 in Mouse Pancreas. Collagen I α1 was detected in perfusion fixed paraffin-embedded sections of mouse pancreas using Rat Anti-Mouse Collagen I α1 Monoclonal Antibody (Catalog # MAB11700) at 5 µg/ml overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using VisUCyte Antigen Retrieval Reagent-Basic (Catalog # VCTS021). Tissue was stained using the HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005) and counterstained with hematoxylin (blue). Specific staining was localized to the cytoplasm. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

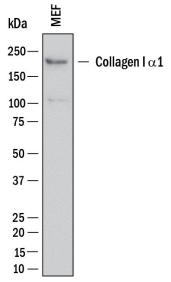
Immunocytochemistry



Positive (3T3-L1 cells) Negative (RAW264.7 cells)

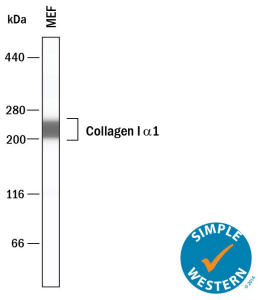
Detection of Collagen I α1 in 3T3-L1 cells. Collagen I α1 was detected in immersion fixed 3T3-L1 mouse embryonic fibroblast adipose-like cell line (Positive) and absent in RAW 264.7 mouse monocyte/macrophage cell line (Negative) using Rat Anti-Mouse Collagen I α1 Monoclonal Antibody (Catalog # MAB11700) at 8 µg/ml for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Rat IgG Secondary Antibody (red; Catalog # NL013) and counterstained with DAPI (blue). Specific staining was localized to the cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.

Western Blot



Detection of Mouse Collagen I α1 by Western Blot. Western Blot shows lysates of MEF mouse embryonic feeder cells. PVDF membrane was probed with 1 µg/ml of Rat Anti-Mouse Collagen I α1 Monoclonal Antibody (Catalog # MAB11700) followed by HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005). A specific band was detected for Collagen I α1 at approximately 220 kDa (as indicated). This experiment was conducted under reducing conditions and using Western Blot Buffer Group 1.

Simple Western



Detection of Mouse Collagen I α1 by Simple Western™. Simple Western lane view shows lysates of MEF mouse embryonic feeder cells, loaded at 0.1 mg/ml. A specific band was detected for Collagen I α1 at approximately 226 kDa (as indicated) using 10 µg/ml of Rat Anti-Mouse Collagen I α1 Monoclonal Antibody (Catalog # MAB11700) followed by 1:50 dilution of HRP-conjugated Anti-Rat IgG Secondary Antibody (Catalog # HAF005) in Milk-free Antibody Diluent (Catalog # 043-524). This experiment was conducted under reducing conditions and using the 66-440 kDa separation system.

PREPARATION AND STORAGE

Reconstitution	Reconstitute lyophilized material at 0.2 mg/ml in sterile PBS. For liquid material, refer to CoA for concentration.
Shipping	Lyophilized product is shipped at ambient temperature. Liquid small pack size (-SP) is shipped with polar packs. Upon receipt, store immediately at the temperature recommended below.
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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