

Visualization of Global Collagen in FFPE Using a Hydroxyproline Antibody

Introduction

Hydroxyproline is a major component of, and is expressed almost exclusively in, collagen. Measurement of hydroxyproline content in tissues is a commonly used means of determining collagen content. Classical assays used to quantify hydroxyproline in tissue require tedious sample processing steps that utilize hazardous, toxic, and highly reactive chemicals that require special handling and disposal^{1,2}. Here, we describe a means of visualizing hydroxyproline in paraffin-embedded tissues using a routine immunohistochemical assay that is easily implemented by any laboratory. Using our hydroxyproline antibody in the IHC assay, the user can detect global collagen in tissue, providing contextual information that is lost in traditional hydroxyproline assays. Additionally, the hydroxyproline antibody can be combined with other antibodies to enable detection of other markers of interest, something that is not easily achievable with the Sirius Red stain, a conventional means of global collagen detection³.

Methods

Single Stain: FFPE tissues were deparaffinized and rehydrated, subjected to antigen retrieval, then incubated overnight at 4°C with Hydroxyproline Antibody #73812 or COL1A1 (E8F4L) Rabbit mAb #72026 diluted in SignalStain® Antibody Diluent #8112. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Rabbit) #8114 and SignalStain® DAB Substrate Kit #8059.

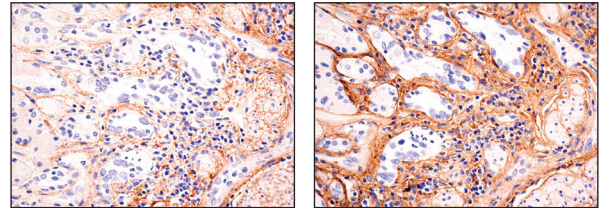
Dual Stain: Deparaffinized and rehydrated sections were subjected to antigen retrieval, then incubated 1 hour at room temperature with E-Cadherin (4A2) Mouse mAb #14472 and Hydroxyproline Antibody #73812 diluted in SignalStain® Antibody Diluent #8112. Detection was performed using SignalStain® Boost IHC Detection Reagent (HRP, Mouse) #8125 and DAB Substrate Kit #8059, followed by SignalStain® Boost IHC Detection Reagent (AP, Rabbit) #18653 and SignalStain® Vibrant Red Alkaline Phosphatase Substrate Kit #76713. C57BL/6NTac mice (Taconic Biosciences) were fed a diet with 5% fat (control mice) or Amylin liver NASH (AMLN) diet (Diet # D09100310i) for 28 weeks to induce NASH.

CONCLUSIONS

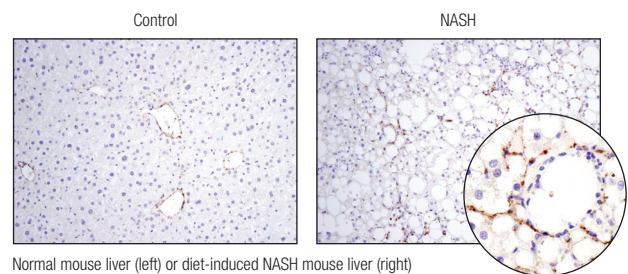
- Hydroxyproline Antibody enables visualization of global collagen in FFPE tissue samples by a routine IHC assay.
- Hydroxyproline Antibody allows for species-independent detection of global collagen in FFPE tissues.
- Hydroxyproline Antibody can be combined with other antibodies to enable visualization of collagen, along with other markers of interest.

References

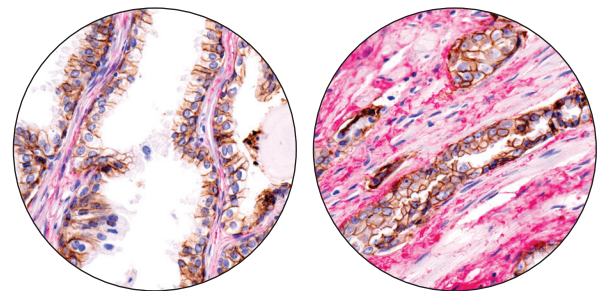
1. Neuman R.E., and Logan M.A. The determination of hydroxyproline. *J Biol Chem* 1950 184, 299-306
2. Cissell DD, Link JM, Hu JC, Athanasiou KA. A Modified Hydroxyproline Assay Based on Hydrochloric Acid in Ehrlich's Solution Accurately Measures Tissue Collagen Content. *Tissue Eng Part C Methods*. 2017 Apr;23(4):243-250
3. Wing-Kin Syn, Kolade M Agboola, Marzena Swiderska, Gregory A Michelotti, Evaggelia Liaskou, Herbert Pang, Guanhua Xie, George Philips, Isaac S Chan, Gamze F Karaca, Thiago de Almeida Pereira, Yuping Chen, Zhiyong Mi, Paul C Kuo, Steve S Choi, Cynthia D Guy, Manal F Abdelmalek, Anna Mae Diehl. NKT-associated hedgehog and osteopontin drive fibrogenesis in non-alcoholic fatty liver disease. *Gut* 2012;61:1323e1329
4. Huan Qiao, Jason Bell, Saul Juliao, Lying Li, and James M. May. Ascorbic Acid Uptake and Regulation of Type I Collagen Synthesis in Cultured Vascular Smooth Muscle Cells. *J Vasc Res*. 2009; 46(1): 15-24



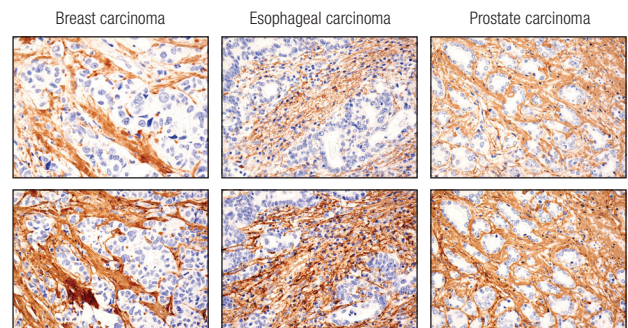
Human fibrosis of the stomach stained with Hydroxyproline Antibody #73812 (left) or COL1A1 (E8F4L) Rabbit mAb #72026 (right).



Normal mouse liver (left) or diet-induced NASH mouse liver (right) stained with Hydroxyproline Antibody #73812.

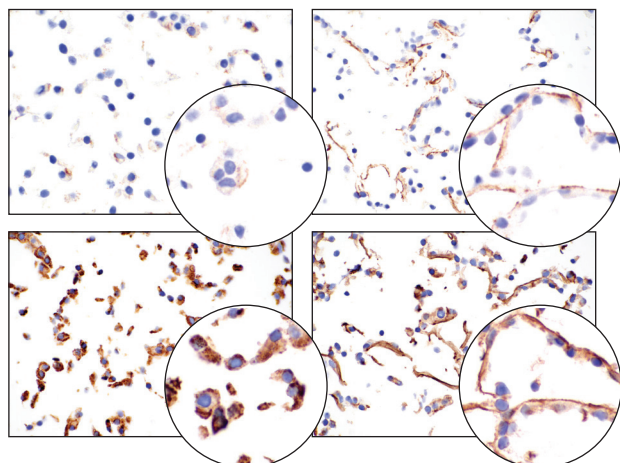


Dual immunohistochemical staining of paraffin-embedded human prostate carcinoma, 2 regions of interest, using E-Cadherin (4A2) Mouse mAb #14472 (brown) and Hydroxyproline Antibody #73812 (red).

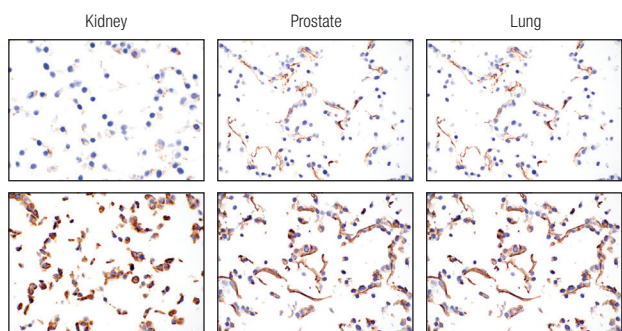


Human tumor tissues stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb #72026 (bottom).

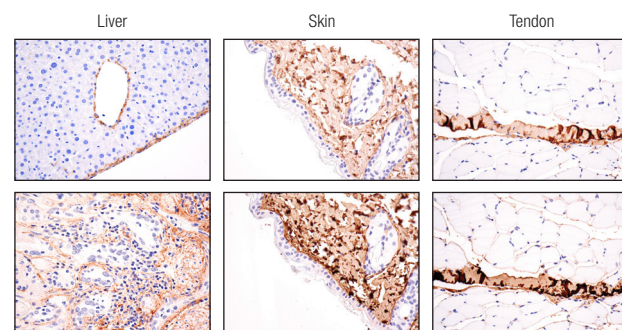
Visualization of Global Collagen in FFPE Using a Hydroxyproline Antibody



A-10 cells, control (left) or treated with dehydroxyascorbic acid 4 (100 μ M, daily for 3 days; right) stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb #72026 (bottom).



Normal human tissues stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb #72026 (bottom).



Normal mouse tissues stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb #72026 (bottom).

Related Products

ANTIBODIES	APPLICATIONS	REACTIVITY
14472 E-Cadherin (4A2) Mouse mAb	WB, IP, IHC, IF, F	H, M, R
3195 E-Cadherin (24E10) Rabbit mAb	WB, IHC, IF, F	H, M
13116 N-Cadherin (D4R1H) XP® Rabbit mAb	WB, IP, IHC, IF	H, M
13255 Claudin-1 (D5H1D) XP® Rabbit mAb	WB, IP, IHC	H, Dg
42818 COL11A1 Antibody	WB	H
72026 COL1A1 (E8F4L) Rabbit mAb	WB, IP, IHC, IF	H
39952 COL1A1 (E6A8E) Rabbit mAb	WB, IP, IF-IC	H
91144 COL1A1 (E8I9Z) Rabbit mAb	WB, IP	H, M, R
84336 COL1A1 Antibody	WB, IP	H, M, R
30565 COL3A1 Antibody	WB, IP	H
37304 COL5A1 Antibody	WB	H
70458 COL11A1 (E607R) Rabbit mAb	WB	H
96321 COL11A1 (E6X3Y) Rabbit mAb	WB, IP, F	H
50273 COL4A1 Antibody	WB	H
26836 Fibronectin/FN1 (E5H6X) Rabbit mAb	WB, IP, IHC, IF	H
36169 HIF-1 α (D1S7W) XP® Rabbit mAb	WB, IP, IF, ChIP, F	H, M, Mk
73812 Hydroxyproline Antibody	IHC	All
58135 LOX (D8F2K) Rabbit mAb	WB	H, M
99680 LOXL2 (E3P7Y) Rabbit mAb	WB	H
54376 MMP-1 (E9S9N) Rabbit mAb	WB, IP	H
87809 MMP-2 (D204T) Rabbit mAb	WB, IP	H, M
40994 MMP-2 (D4M2N) Rabbit mAb	WB, IP, IHC-P, IF-IC	H
14351 MMP-3 (D7F5B) Rabbit mAb	WB	H, R
3801 MMP-7 (D4H5) XP® Rabbit mAb	WB, IHC-P	M, R
13667 MMP-9 (D603H) XP® Rabbit mAb	WB, IHC-P, F	H
94808 MMP-13 Antibody	WB	H, (M, R, Mk)
69926 MMP-13 (E4W3T) Rabbit mAb	WB	H
13130 MT1-MMP (D1E4) Rabbit mAb	WB	H, M
91771 Periostin Antibody	WB, IP	H
48938 α -Smooth Muscle Actin (1A4) Mouse mAb (IF Formulated)	IF-F	H, M, R
56856 α -Smooth Muscle Actin (1A4) Mouse mAb (IHC Formulated)	IHC-P	H, M, R
19245 α -Smooth Muscle Actin (D4K9N) XP® Rabbit mAb	WB, IP, IHC-P, IF-IC, IF-F	H, M, R
8725 SPARC (D10F10) Rabbit mAb	WB, IHC	H, M
12221 Tenascin C (D16C4) Rabbit mAb	WB, IP	H, M, R
8946 TIMP1 (D10E6) Rabbit mAb	WB	H, Mk
5738 TIMP2 (D18B7) Rabbit mAb	WB	H, M, Mk
5673 TIMP3 (D74B10) Rabbit mAb	WB	H, M, R
5741 Vimentin (D21H3) XP® Rabbit mAb	WB, IHC, IF, F	H, M, R, Mk

RELATED KITS

33437 ECM Profiling Antibody Sampler Kit
9782 Epithelial-Mesenchymal Transition (EMT) Antibody Sampler Kit
13430 Focal Adhesion Protein Antibody Sampler Kit
73959 Matrix Remodeling Antibody Sampler Kit
77397 TGF- β Fibrosis Pathway Antibody Sampler Kit

© 2020 Cell Signaling Technology, Inc. All trademarks are the property of their respective owners. U.S. Patent No. 7,429,487, foreign equivalents, and child patents deriving therefrom.
For the most up-to-date trademark information, please visit www.cellsignal.com/trademarks

Learn more at:
www.cst-science.com/hydroxyproline

