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 detection[3].

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 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 FPPE 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

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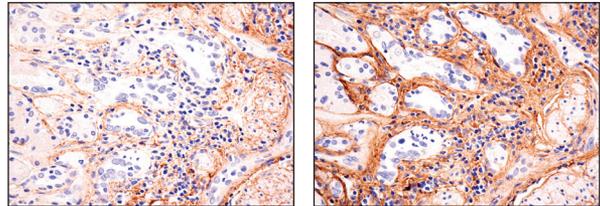
Human fibrosis of the stomach stained with Hydroxyproline Antibody #73812 (left) or COL1A1 (E8F4L) Rabbit mAb (right).

Control   NASH
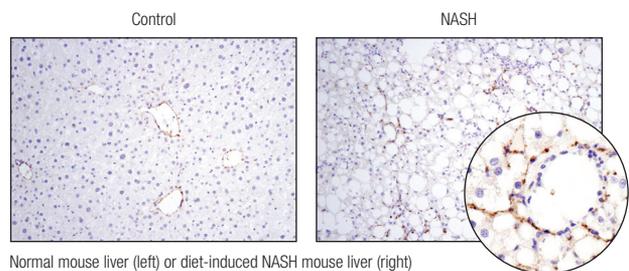
Normal mouse liver (left) or diet-induced NASH mouse liver (right) stained with Hydroxyproline Antibody #73812.
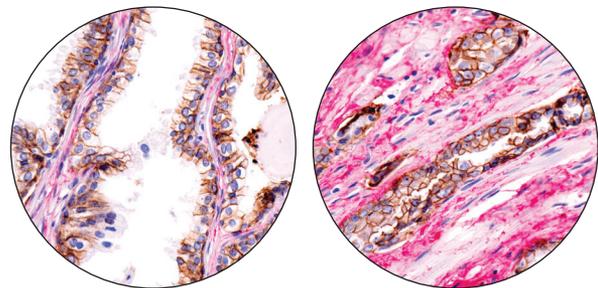
Dual immunohistochemical staining of paraffin-embedded human prostate carcinoma, 2 regions of interest, using E-cadherin (brown) and Hydroxyproline (red).
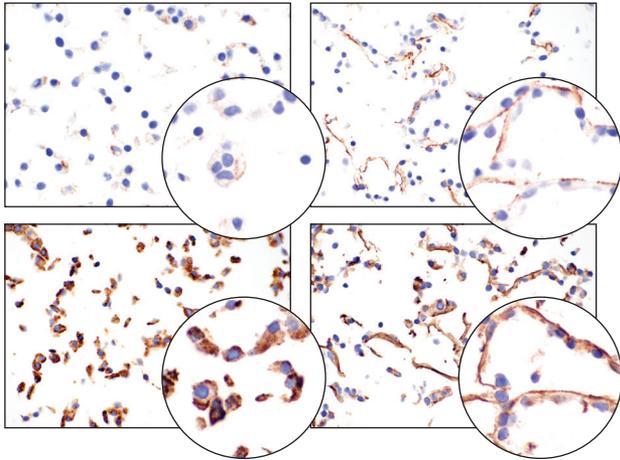

Breast carcinoma   Esophageal carcinoma   Prostate carcinoma
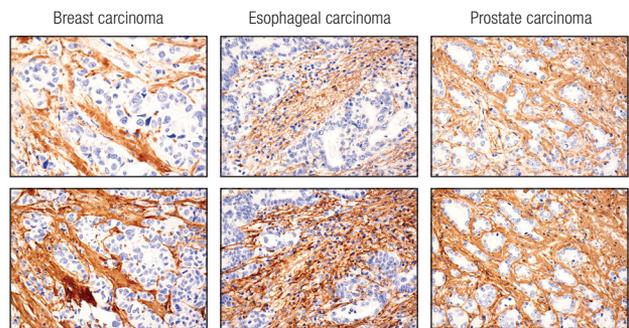
Human tumor tissues stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

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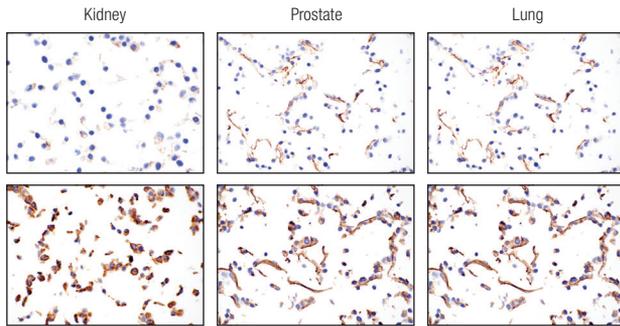
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A-10 cells, control (left) or treated with dehydroxyascorbic acid 4 (100 μM, daily for 3 days; right) stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

Kidney   Prostate   Lung

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

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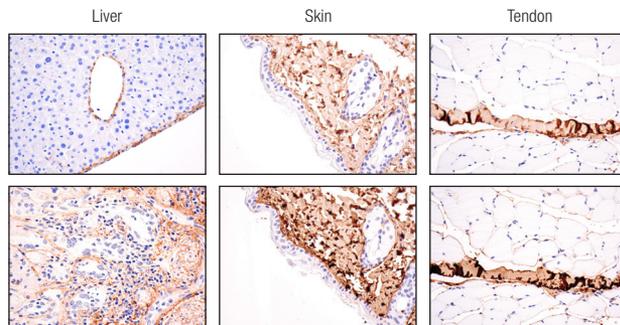
Normal mouse tissues stained with Hydroxyproline Antibody #73812 (top) or COL1A1 (E8F4L) Rabbit mAb (bottom).

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ANTIBODIES		APPLICATIONS	REACTIVITY
14472	E-Cadherin (4A2) Mouse mAb	WB, IP, IHC ,IF, F	H, M, R
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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
39952	COL1A1 (E6A8E) Rabbit mAb	WB, IP, IF-IC	H
91144	COL1A1 (E8I9Z) XP® Rabbit mAb	WB, IP, IHC-P	H
84336	COL1A1 Antibody	WB, IP	H, M, R
30565	COL3A1 Antibody	WB,IP	H
37304	COL5A1 Antibody	WB	H
70458	COL11A1 (E6O7R) 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-1α (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 (D2O4T) 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 (D6O3H) 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
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