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AF5110-1 Monoclonal Antibody to Prolyl-4-Hydroxylase beta - Purified

Alternate names: Fibroblast marker, P4HB, Prolyl 4-hydroxylase beta

Quantity: 0.1 mg

Concentration: 0.2 mg/ml (after reconstitution)

Background: Collagen Prolyl 4-Hydroxylases (P4H) play an essential role in the synthesis of all collagens. Two alpha and two beta subunits assemble into P4H tetramers in which protein disulfide isomerase (PDI) acts as the beta subunit.
P4HB, Prolyl 4 hydroxylase subunit beta, is a multifunctional and highly abundant enzyme that belongs to the protein disulfide isomerase family. When present as a tetramer consisting of two alpha and two beta subunits, this enzyme is involved in hydroxylation of prolyl residues in preprocollagen. This enzyme is also a disulfide isomerase containing two thioredoxin domains that catalyze the formation, breakage and rearrangement of disulfide bonds.

Host / Isotype: Mouse / IgG1

Recommended Isotype Controls: SM20P (for use in rat samples), AM03095PU-N

Clone: 6-9H6

Immunogen: Prolyl-4-Hydrolase beta.
The epitope is not further characterized.

Format:
State: Lyophilized purified IgG fraction
Purification: Affinity Chromatography on Protein A
Buffer System: PBS, pH 7.2
Preservatives: 0.09% Sodium Azide
Stabilizers: 0.5% BSA
Reconstitution: Restore in 0.5 ml double distilled water

Applications:
ELISA.
Western blot: 2-5 µg/ml (See also Bai, Y. et al. 1986).
Immunofluorescence.
Immunohistochemistry on Frozen Sections: 1 µg/ml (1/200) (See Protocols below).
Immunohistochemistry on Paraffin Sections: 10 µg/ml (1/20), Microwave pretreatment for antigen retrieval is recommended (See Protocols below).
Recommended Positive Control: Rat skin.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: This antibody specifically reacts with the beta-subunit of Rat P4H (Fibroblast Marker) from the skin of newborn Rat.
Does not react with Mouse according to customer information.

Species Reactivity: **Tested:** Rat.

Add. Information: Has been used successfully as a marker antibody for staining Fibroblasts in Immunohistochemistry applications.

Storage:

Prior to reconstitution store at 2-8°C.
Following reconstitution store undiluted at 2-8°C for one month
or (in aliquots) at -20°C for longer.
Avoid repeated freezing and thawing.
Shelf life: one year from despatch.

Product Citations:

Purchased from Acris:

1. Zheng W, Christensen LP, Tomanek RJ. Differential effects of cyclic and static stretch on coronary microvascular endothelial cell receptors and vasculogenic/angiogenic responses. Am J Physiol Heart Circ Physiol. 2008 Aug;295(2):H794-800. doi: 10.1152/ajpheart.00343.2008. Epub 2008 Jun 27. PubMed PMID: 18586890.
2. Gruh I, Beilner J, Blomer U, Schmiedl A, Schmidt-Richter I, Kruse ML, et al. No evidence of transdifferentiation of human endothelial progenitor cells into cardiomyocytes after coculture with neonatal rat cardiomyocytes. Circulation. 2006 Mar 14;113(10):1326-34. Epub 2006 Mar 6. PubMed PMID: 16520414.
3. Hahn JY, Cho HJ, Bae JW, Yuk HS, Kim KI, Park KW, et al. Beta-catenin overexpression reduces myocardial infarct size through differential effects on cardiomyocytes and cardiac fibroblasts. J Biol Chem. 2006 Oct 13;281(41):30979-89. Epub 2006 Aug 18. PubMed PMID: 16920707.
4. Koleganova N, Piecha G, Ritz E, Gross ML. Calcitriol ameliorates capillary deficit and fibrosis of the heart in subtotally nephrectomized rats. Nephrol Dial Transplant. 2009 Mar;24(3):778-87. doi: 10.1093/ndt/gfn549. Epub 2008 Oct 1. PubMed PMID: 18829613.
5. Kapur NK, Deming CB, Kapur S, Bian C, Champion HC, Donahue JK, et al. Hemodynamic modulation of endocardial thromboresistance. Circulation. 2007 Jan 2;115(1):67-75. Epub 2006 Dec 26. PubMed PMID: 17190863.
6. Haylor J, Schroeder J, Wagner B, Nutter F, Jestin G, Idée JM, et al. Skin gadolinium following use of MR contrast agents in a rat model of nephrogenic systemic fibrosis. Radiology. 2012 Apr;263(1):107-16. doi: 10.1148/radiol.12110881. Epub 2012 Feb 17. PubMed PMID: 22344402.
7. Nishida, E;Miyaji, H;Kato, A;Takita, H;Iwanaga, T;Momose, T;Ogawa, K;Murakami, S;Sugaya, T;Kawanami, M;2016Graphene oxide scaffold accelerates cellular proliferative response and alveolar bone healing of tooth extraction socket. Int J Nanomedicine 2016. 2265-77, 11. PubMed PMID: 27307729.
8. Edanami, N;Yoshiba, N;Ohkura, N;Takeuchi, R;Tohma, A;Noiri, Y;Yoshiba, K;2017Characterization of Dental Pulp Myofibroblasts in Rat Molars after PulpotomyJ Endod 2017. PubMed PMID: 28527846.
9. Murakami, S;Miyaji, H;Nishida, E;Kawamoto, K;Miyata, S;Takita, H;Akasaka, T;Fugetsu, B;Iwanaga, T;Hongo, H;Amizuka, N;Sugaya, T;Kawanami, M. Dose effects of beta-tricalcium phosphate nanoparticles on biocompatibility and bone conductive ability of three-dimensional collagen scaffolds. Dent Mater J 2017. PubMed PMID: 28450672.

General Readings:

1. Bai Y, Muragaki Y, Obata K, Iwata K, Ooshima A. Immunological properties of monoclonal antibodies to human and rat prolyl 4-hydroxylase. J Biochem. 1986 Jun;99(6):1563-70. PubMed PMID: 3017922.
2. Okuno M, Muto Y, Kato M, Moriwaki H, Noma A, Tagaya O, et al. Changes in serum and hepatic levels of immunoreactive prolyl hydroxylase in two models of hepatic

fibrosis in rats. J Gastroenterol Hepatol. 1991 May-Jun;6(3):271-7. PubMed PMID: 1655096.

Protocols:

Frozen Sections

Incubations are done at RT. Water is of double distilled or comparable quality.
1. Fix fresh frozen sections in ice-cold acetone for 10 min
2. Block endogenous peroxidase with 100ml 0.15M Sodium Azide / 0.15% H_2O_2 in PBS
3. Wash in PBS
4. Block with 10% Normal Goat Serum (Jackson #005-000-121) for 30 min in a humid chamber
5. Incubate with primary antibody *Cat.-No* AF5110-1 at 1 µg/ml for 1 hour in a humid chamber
6. Wash in PBS
7. Incubate with secondary antibody (peroxidase-conjugated Goat Anti Mouse IgG (H+L), f.e. *Cat.-No* R1253HRP) at a 1/200 dilution for 1 hour in a humid chamber
8. Wash in PBS
9. Incubate with AEC substrate (3-Amino-9-Ethylcarbazol) for 12 min
10. Wash in PBS
11. Counterstain with Mayer's hemalum.

Paraffin Sections

Incubations are done at RT. Water is of double distilled or comparable quality.
1. Rehydrate paraffin sections
2. Put the slides in a cuvette with 250 ml 0.01 M citrate buffer pH 6.0
3. Heat the slides in a microwave oven for 2 x 7 min and 700Watt
4. Leave the slides in the buffer for 20 min
5. Block endogenous peroxidase with 1%H_2O_2 in water
6. Wash in PBS
7. Block with 10% Normal Goat Serum (Jackson #005-000-121) for 30 min in a humid chamber
8. Incubate with primary antibody *Cat.-No* AF5110-1 at 10 µg/ml for 1 hour in a humid chamber
9. Wash in PBS
10. Incubate with secondary antibody (peroxidase-conjugated Goat Anti Mouse IgG (H+L), f.e *Cat.-No* R1253HRP) at a 1/200 dilution for 1 hour in a humid chamber
11. Wash in PBS
12. Incubate with AEC substrate (3-Amino-9-Ethylcarbazol) for 12 min
13. Wash in PBS
14. Counterstain

Immunofluorescence

1. Wash with PBS
2. Fixation using 3.7% Formaldehyde for 10 min
3. Blocking using PBS containing 0.2% Triton X-100 and 1% BSA for 10 min
4. Incubation with 1. Ab (Mouse anti-Rat anti Prolyl-4-Hydroxylase-beta *Cat.-No* AF5110-1) for 1 hour
5. 3x wash for 5 min using PBS containing 0.2% Triton X-100

6. Inkubation wth 2. Ab (FITC-labeled Goat anti-Mouse, *Cat.-No* R1253F) for 1 hour
7. 3x wash for 5 min using PBS containing 0.2% Triton X-100.

Pictures:

Figure 2. Staining of Rat spleen paraffin sections using Mouse anti Rat Prolyl-4-Hydroxylase beta (fibroblast marker) antibody 6-9H6 (Cat.-No AF5110-1).

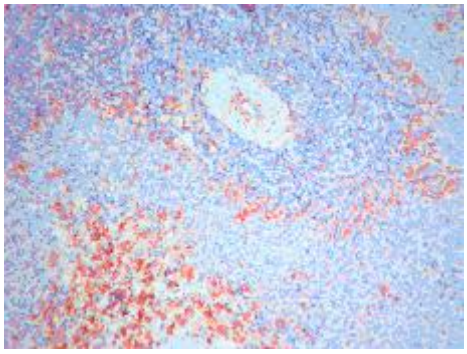

Figure 4. Immunofluorescence of Rat fibroblasts using AF5110-1 dilution 1/50 *(Dr. Patrick Maier Universitätsklinik für Strahlentherapie und Radioonkologie, Mannheim. Universität Heidelberg)*

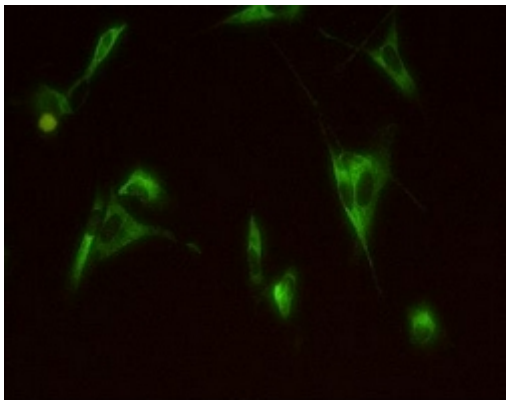

Figure 3. Immunofluorescence of Rat fibroblasts using AF5110-1 dilution 1/100 *(Dr. Patrick Maier, Universitätsklinik für Strahlentherapie und Radioonkologie, Mannheim. Universität Heidelberg)*

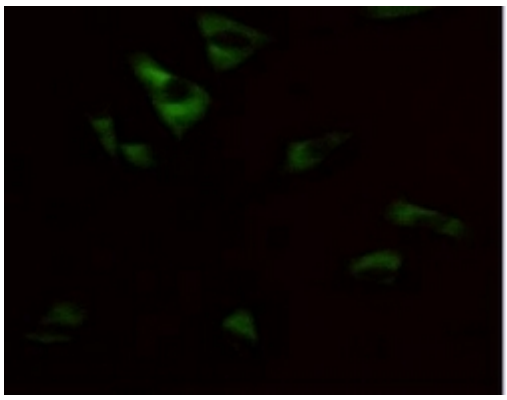

Figure 1. Staining of frozen Rat skin sections using Mouse anti Rat Prolyl-4-Hydroxylase beta (fibroblast marker) antibody 6-9H6 (Cat.-No AF5110-1).