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Monoclonal Antibody to Prolyl 4-hydroxylase subunit beta - Purified

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

Catalog No.: AF0910-1

Quantity: 0.1 mg

Concentration: 0.2 mg/ml (after reconstitution)

Background: Collagen prolyl 4-hydroxylases (P4Hs) 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.
The three dimensional structure of many extracellular proteins is stabilized by the formation of disulphide bonds. Studies suggest that a microsomal enzyme known as Protein Disulphide Isomerase (PDI) is involved in disulphide-bond formation and isomerization, as well as the reduction of disulphide bonds in proteins. PDI, which catalyses disulphide interchange between thiols and protein dilsulphides, has also been referred to as thiol:protein-disulphide oxidoreductase and as glutathione:insulin transhydrogenase because of its role in reduction of disulphide bonds. The highly conserved sequence Lys-Asp-Glu-Leu (KDEL) is present at the carboxy-terminus of PDI and other soluble endoplasmic reticulum (ER) resident proteins including the 78 and 94 kDa glucose regulated proteins (GRP78 and GRP94 respectively). The presence of carboxy-terminal KDEL appears to be necessary for ER retention and appears to be sufficient to reduce the secretion of proteins from the ER. This retention is reported to be mediated by a KDEL receptor.

Host / Isotype: Mouse / IgG1

Clone: 3-2B12

Immunogen: Native Human merosin M-chain.
The epitope has not been further determined.

Format:
State: Lyophilized purified Ig fraction
Purification: Affinity Chromatography
Buffer System: PBS, pH 7.2, containing 0.5% protease-free BSA, 0.09% Sodium Azide as preservative.
Reconstitution: Restore in 0.5 ml double distilled water

Applications:
Immunohistochemistry on Frozen Sections: 0.25-0.5 µg/ml (1/400 - 1/800).
Immunohistochemistry on Paraffin Sections: 4 µg/ml (1/50). Microwave pretreatment for antigen retrieval is recommended.
Suggested Positive Control: Human skin, tonsil.
Has been described to work in **Western Blots** (Ref.1) showing a band at approx. 60kDa.

Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Clone 3-2B12 reacts with the beta subunit of Human P4H specifically found in fibroblasts.

Species Reactivity: **Tested:** Human

Add. Information: Has been reported to cross react with Rat P4H beta in Western blot and ELISA.
Some users have reported cross reactivities with myoepithelial and urothelial cells.

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. 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.
2. Seibl R, Birchler T, Loeliger S, Hossle JP, Gay RE, Saurenmann T, et al. Expression and regulation of Toll-like receptor 2 in rheumatoid arthritis synovium. Am J Pathol. 2003 Apr;162(4):1221-7. PubMed PMID: 12651614.
3. Zong W, Meyn LA, Moalli PA. The amount and activity of active matrix metalloproteinase 13 is suppressed by estradiol and progesterone in human pelvic floor fibroblasts. Biol Reprod. 2009 Feb;80(2):367-74. doi: 10.1095/biolreprod.108.072462. Epub 2008 Nov 5. PubMed PMID: 18987329.
4. Beyer C, Schramm A, Akhmetshina A, Dees C, Kireva T, Gelse K, et al. β-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. Ann Rheum Dis. 2012 May;71(5):761-7. doi: 10.1136/annrheumdis-2011-200568. Epub 2012 Feb 10. PubMed PMID: 22328737.

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.

Protocols: **Protocol for 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 - Wash in PBS
3. Block with 10% Normal Rabbit Serum for 30 min in a humid chamber
4. Incubate with primary antibody Cat.-No AF0910-1 for 1 hour in a humid chamber - Wash in PBS
5. Incubate with secondary antibody (peroxidase-conjugated Rabbit Anti Mouse IgG Cat.-No R1253HRP), at the recommended dilution for 1 hour in a humid chamber - Wash in PBS
6. Incubate with AEC substrate (3-Amino-9-Ethylcarbazol) for 12 minutes - Wash in PBS
7. Counterstain with Mayer's hemalum.

Protocol for 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 700 Watt
4. Leave the slides in the buffer for 20 min
5. Block endogenous peroxidase with 1% H_2O_2 in water - Wash in PBS

6. Block with 10% Normal Goat Serum for 30 min in a humid chamber
7. Incubate with primary antibody cat.-No AF0910-1 for 1 hour in a humid chamber - Wash in PBS
8. Incubate with secondary antibody (peroxidase-conjugated Goat Anti Mouse IgG (H+L), at a dilution recommended by the manufacturer for 1 hour in a humid chamber - Wash in PBS
9. Incubate with AEC substrate (3-Amino-9-Ethylcarbazol) for 12 minutes - Wash in PBS
10. Counterstain with Mayer's hemalum.

Pictures:

Figure.1: AEC staining of Paraffin Sections of Human tonsills with Mouse Anti Human Prolyl-4-Hydroxylase beta (Fibroblast marker) clone 3-2B12. Cat.-No. AF0910-1