

anti-Col1-^{3/4}C (collagen type I cleavage site) affinity purified rabbit antibody

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immunoGlobe
Antikörpertechnik GmbH

Background Information

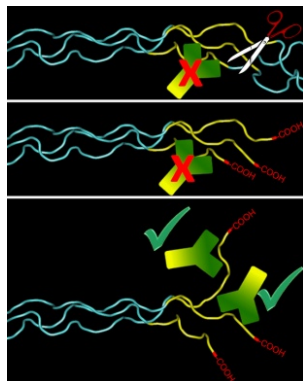
The proteolysis of collagens plays an important role in numerous physiological and pathological situations such as morphogenesis, wound healing, arthritis, arteriosclerosis, and tumor metastasis. Triple helical type I collagens are made up of two $\alpha 1$ (I) and one $\alpha 2$ (I) chains, and are found in skin, tendon, ligament and interstitial tissues. Due to their fibrillary structure native collagens are resistant to most proteases. They are substrates however for certain matrix metalloproteinases (MMPs), which constitute a family of zinc-dependent enzymes catalyzing the degradation of extracellular matrix components [1,2]. Initial MMP-8 dependent cleavage of collagen into the characteristic $3/4$ and $1/4$ fragments has been shown to enable MMP-9 diffusion along the protein helix, with preferential binding to the collagen $3/4$ fragment tail. Finally, untwisting of the helix end results in the local denaturation of the triple helical structure [3].

Antibody preparation and Storage

50 μ g of antibody (250 μ g/ml in PBS with 1 mg/ml BSA and 0.02% [w/v] NaN₃), affinity purified on a synthetic epitope peptide. For repeated use store at 4°C (short term). Stable for one year from date of shipment when stored at -20°C.

Antigen

Synthetic peptide (human sequence) corresponding to the carboxy-terminal end of the N-terminal three quarter collagen fragment (Col1 $3/4$), which results from MT1-MMP, MMP-1, MMP-2, or MMP-8 dependent cleavage of the $\alpha 1$ (I) and $\alpha 2$ (I) chains at the Gly⁷⁷⁵-Ile⁷⁷⁶ bond and Gly⁷⁷⁵-Leu⁷⁷⁶ bond, respectively [1,2].



Model depicting antibody detection of Col1 $3/4$ C.

MMPs cleaving the α chains, create free COOH groups at the C-terminal end of the $3/4$ fragment, which gets untwisted and exposes the antibody epitope. The carboxyl group proper is not part of this epitope. However, there is also a companion antibody available (IG-1266) that requires the free carboxyl group for binding.

Species cross-reactivity

Tested: rat, bovine

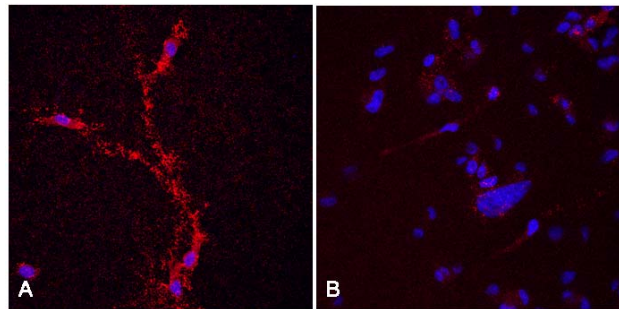
By inference (sequence identity): human, mouse ($\alpha 1$), Chinese hamster ($\alpha 1$), guinea pig, dog, cat, donkey, sheep, pig ($\alpha 2$), chicken

Specificity

The antibody detects collagen α (I) chains after remodelling, e.g. as initiated by matrix metalloprotease dependent cleavage. The antibody epitope is masked in native collagen.

Applications

Immunofluorescence of formaldehyde fixed samples: 2.5-10 μ g/ml. Cell tracks in 3D collagen matrices are best visualized in spheroids (hanging droplet) made from rat tail tendon type I collagen (acid-extracted).



Collagen degradation by human breast cancer MDA-MB-231 cells embedded in a 3D collagen matrix (2.2 mg/ml). Cells have been treated with non-targeting siRNA (A) or siRNA specific for MT1-MMP (B; knock down control) for 48 hours and then transferred into collagen for 24 hours. After fixation (4% PFA at 37°C for 30 min) samples were labeled with **collagen type I cleavage site antibody** diluted 1:100 in PBS (2.5 μ g/ml, 2 h at 4°C). Confocal photomicrograph: Anti-rabbit antibody (red), DNA staining (DAPI; blue).

(Data courtesy of Marie Irondelle & Dr. Philippe Chavrier)

References

- [1] Song F., Wisithphrom K., Zhou J. & Windsor L. J. (2006). Matrix Metalloproteinase Dependent and Independent Collagen Degradation. *Frontiers in Bioscience*, **11**:3100-20.
- [2] Bertini I., Fragai M., Luchinat C., Melikian M., Toccafondi M., Lauer Ja. L. & Fields G. B. (2012). The Structural Basis for Matrix Metalloproteinase 1 Catalyzed Collagenolysis. *J. Am. Chem. Soc.* **134**(4): 2100-2110.
- [3] Rosenblum G., Van den Steen P. E., Cohen S. R., Bitler A., Brand D. D., Opdenakker G. & Sagi I. (2010). Direct Visualization of Protease Action on Collagen Triple Helical Structure. *PLoS ONE* **5**(6): e11043.

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