a n t i - C o l 1 - ¾C
(collagen type I cleavage site)
affinity purified rabbit antibody

Product: #0217-025 **Lot: #2363** **Revision: 21/04/09**

immunoGlobe
Antikörpertechnik GmbH

Background Information

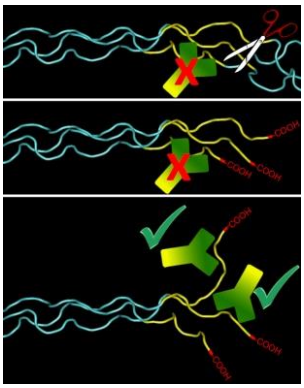
The proteolysis of collagens plays an important role in numerous physiological and pathological situations such as morphogenesis, wound healing, arthritis, arteriosclerosis, and tumor metastasis. Triple helical type I collagens are made up of two α 1 (I) and one α 2 (I) chains, and are found in skin, 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 [23,25]. Initial MMP-8 dependent cleavage of collagen into the characteristic ¾ and ¼ fragments has been shown to enable MMP-9 diffusion along the protein helix, with preferential binding to the collagen ¾ fragment tail. Finally, untwisting of the helix end results in the local denaturation of the triple helical structure [24].

Antibody preparation and Storage

25 µg of antibody (250 µg/ml in PBS with 1 mg/ml BSA and 0.02% [w/v] NaN_3), 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 ¾), which results from MT1-MMP, MMP-1, MMP-2, or MMP-8 dependent cleavage of the α 1 (I) and α 2 (I) chains at the Gly^{775}–Ile^{776} bond and Gly^{775}–Leu^{776} bond, respectively [23,25].



Model depicting antibody detection of Col1 ¾C. MMPs cleaving the α chains, create free COOH groups at the C-terminal end of the ¾ 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 (α 1), Chinese hamster (α 1), guinea pig, dog, cat, donkey, sheep, pig (α 2), and chicken

Specificity

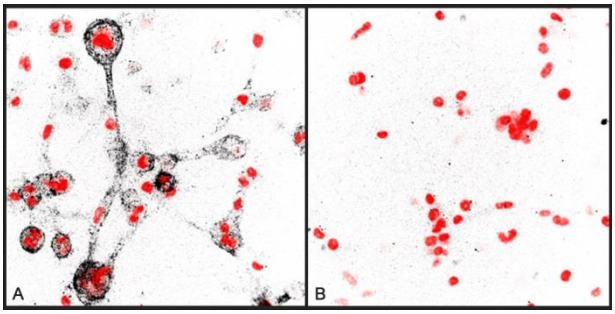
The antibody detects collagen α (I) chains after remodeling, e.g. as initiated by matrix metallo-protease dependent cleavage. The antibody epitope is masked in native collagen.

Applications

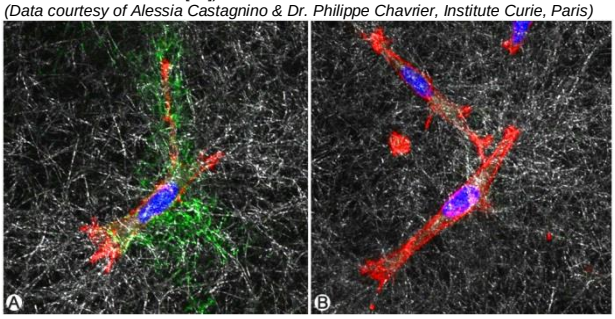
Immunofluorescence of formaldehyde fixed samples: 0.5-10 µg/ml. Cell tracks in 3D collagen matrices are best visualized in spheroids (hanging droplet) made from rat tail tendon type I collagen (acid-extracted).

Western Blotting*: See Mezawa et al. (2016) [11].

* Not tested in-house, but reported in the literature.



Collagen degradation by human MDA-MB-231 breast cancer cells embedded in a 3D type I collagen matrix (2.5 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 labelled with **collagen type I cleavage site antibody** diluted 1:100 in PBS (2.5 µg/ml, 2 h at 4°C; lot #1574-5.1). Confocal photomicrograph: Anti-rabbit antibody (black in the inverted image), nuclei were stained with DAPI (red). For details see Monteiro et al., 2013 [22]).
(Data courtesy of Alessia Castagnino & Dr. Philippe Chavrier, Institute Curie, Paris)



Collagen degradation by human HT1080 fibrosarcoma cells migrating overnight at 37°C in a 3D type I bovine collagen matrix (1.7 mg/ml) in the presence (B, control) or absence (A) of 5 µM matrix metalloproteinase inhibitor GM6001 (both in medium and in the collagen matrix). After fixation (4% PFA) samples were labelled with **collagen type I cleavage site antibody** diluted 1:25 (10 µg/ml, 2 h at 4°C; lot #1574-5.1). Confocal photomicrograph: Alexa 647 goat-anti-rabbit antibody (cleaved collagen, green), DAPI stain (nuclei, blue), phalloidin 568 (F-actin, red), internal reflection (collagen, white/grey). *(Data courtesy of Mariska Kea-te Lindert, Dr. Katarina Wolf & Dr. Peter Friedl, Radboud University Medical Centre, Nijmegen).*

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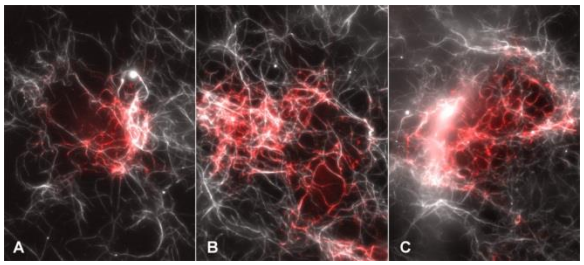

Collagen degradation by human MDA-MB-231 breast cancer cells grown on a rat type I collagen matrix (acid-extracted, 2.2 mg/ml, Corning) for 1 hour. After fixation (4% PFA at 37°C for 30 min) samples were labelled with **collagen type I cleavage site antibody** diluted in PBS (2 h at 4°C). Confocal photomicrograph: Anti-rabbit antibody in red pseudo-color, fibrillar collagen in grey tone. Antibody dilution 1:200 (1.25 µg/ml) in **A** (lot #1574-5.1) and **B** (lot #2363), and 1:500 (0.5 µg/ml) in **C** (lot #2363).
(Data courtesy of Dr. David Remy & Dr. Philippe Chavrier, Institute Curie, Paris)

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