

Product Name

Monoclonal Mouse
Anti-digested human type I collagen (Sigma-Aldrich, C5483)
Immunoglobulin, clone 2G8

CAT No.

MQ3.101-100

Size

100 µg



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Intended use

This product is for research use only. NOT for use in diagnostic or therapeutic procedures.

Mouse monoclonal to collagenase-digested human type I collagen, is intended for use in ELISA, immunoblotting or immuno histochemistry (IHC).

Reagent provided

The antibody is present in 100 mM Tris-HCl, pH 8.0; 50% (v/v) glycerol; 0.05% sodium azide (NaN₃).

Antibody concentration: 2 mg/ml

Isotype

IgM

Immunogen

Human type I collagen (Sigma-Aldrich, C5483), digested with Collagenase from *Clostridium histolyticum* (Sigma-Aldrich, C0773).

Synonyms for Collagenase from *Clostridium histolyticum*; Clostridiopeptidase A

Specificity

In ELISA, this antibody is approximately 2 times more reactive towards digested than intact type I collagen.

In IHC (NOTE: FROZEN SECTIONS ONLY, Not reactive on paraffin-embedded tissue) this antibody, compared to collagen type 1, preferentially recognizes collagenase-digested human type I collagen (figure 1). There is some cross reactivity with human epidermal keratinocytes. No cross reactivity has been detected with mouse collagen.

In immunoblotting (not reducing conditions), the antibody reacts with both intact as digested collagen type 1 (figure 2). The antibody doesn't cross react with human type IV collagen.

Purity

Ammonium sulphate precipitated and DEAE purified.

Precautions

1. For professional users.
2. This product contains sodium azide (NaN₃), a chemical highly toxic in pure form. At product concentration, though not classified as hazardous, sodium azide may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.
3. As with any product derived from biological sources, proper handling procedures should be used.
4. The Product may be used in different techniques and in combination with different sample types and materials, therefore each individual laboratory should validate the test system applied.

Preparation of the antibody

Use antibody as supplied.

Storage instructions

Store at -20°C.

Dilution guidelines

ELISA: 1:(1000 x F) – 1:(2000 x F).

Immunoblotting: 1:(200 x F)

IHC: 1:(100 x F)

Other applications: since applications vary, you should determine the optimum working dilution of the product that is appropriate for your specific need.

For the value of the multiplication factor F, see label on vial.

Unless the stability in the actual test system has been established, it is recommended to dilute the product immediately before use.

Relevance

Collagens play a dominant role in maintaining the structural integrity of organs and tissues (1), but they also regulate cell polarity, migration, survival and phenotype, and provide diffusible signaling molecules following breakdown (2). Within the family of collagens that currently comprises 21 members, type I collagen is included in the group of fibril-forming collagens based on its structural and functional features. Type I collagen is ubiquitous in all vertebrates and is among the largest and most complex of all macromolecules. It accounts for 80-90% of all collagenous proteins in skin (3). The helical trimeric molecules of type I collagen comprise two α1(I) chains and one α2(I) chain, encoded by two separate genes Col(I)α1 and Col(I)α2, respectively (3). Matrix metalloproteinase (MMP)-1,-8, -13 and -14 cleave the peptide bonds between residues Gly775 and Ile776 of the α1(I) chain and Gly775 and Leu776 of the α2(I) chains, resulting in ¼ and ¾ fragments.

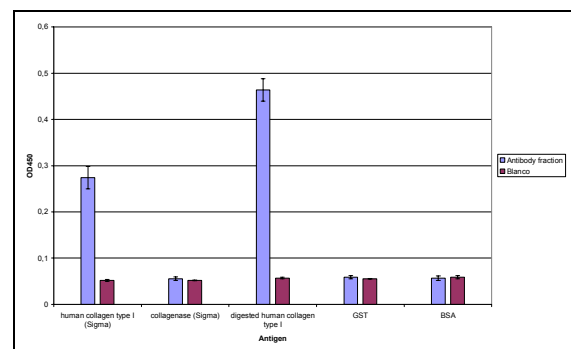


Figure 1: Specificity of anti-digested human type I collagen (Sigma-Aldrich, C5483), determined by ELISA. Antibody fraction (2mg/ml) 1800X diluted in MPBST. Antibody was tested on various recombinant protein substrates i.e. Human type I collagen (Sigma-Aldrich, C5483), GST (pGEX-2T) and BSA, 98% (Sigma).

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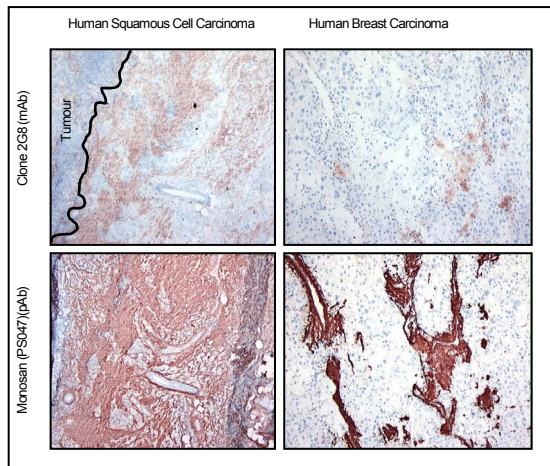


Figure 2: Frozen 4µm sections of human squamous cell carcinoma and human breast carcinoma where stained with anti-Collagenase Digested Human Type I collagen (2G8) (dilution 1:100). Goat anti-Mouse-HRP was used as secondary antibody and developed with 3-amino-9-ethylcarbazole (AEC) according to standard procedures. Digested collagen is shown as a red stain. As a control for intact type I collagen a polyclonal antibody (PS047) from Monosan was used on serial sections. In immunohistochemistry, the 2G8 antibody predominantly recognizes digested type I collagen.

References

1. Vuorio and Crombrugge. The family of collagen genes. *Annu. Rev. Biochem.* 1990;59: 837-872
2. Egeblad and Werb. New functions for the matrix metalloproteinases in cancer progression. *Nat. Rev. Cancer.* 2002;2: 161-174
3. Kielty et al. The Collagen family: Structure, assembly, and organization in the extracellular matrix. *In* Connective tissue and its inheritable disorders. P.M. Royce and B. Steinmann, editors. Wiley-Liss, New York. 1993; 103-147.

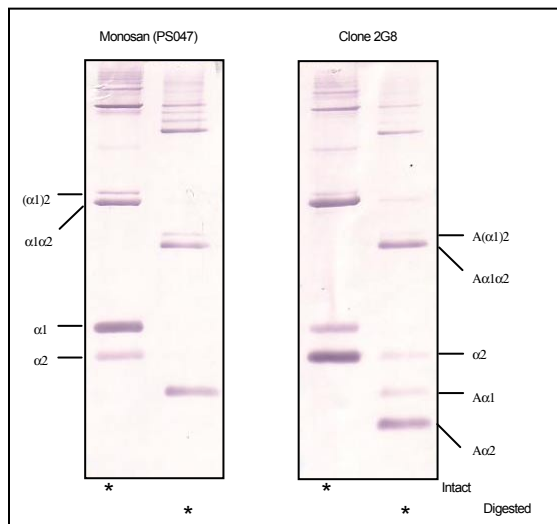


Figure 3: 1µg of intact or matrix metalloproteinase (MMP)-1 (Chemicon) digested human type I collagen was loaded in each lane of a 6% SDS-Page gel under non-reducing conditions which, after electrophoresis, was subjected to Western blotting. The blots were incubated with the Anti Collagenase Degraded Human Type I collagen (2G8) antibody from ModiQuest (right panel) or with the polyclonal antibody (PS047) from Monosan (left panel). Secondary antibodies used were alkaline phosphatase conjugated rabbit-anti-mouse-Ig and alkaline phosphatase conjugated swine-anti-rabbit-Ig, respectively. The human collagen type I $\alpha 1$ and $\alpha 2$ chains are indicated. Capital A in front of the chains indicates digested forms.