コスモ・バイオ株式会社

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

CAT No.
MQ3.101-100

Size
100 µg

POSTAL ADDRESS Schoutstraat 58
6525 XV Nijmegen
The Netherlands

TELEPHONE +31 (0) 24 – 361 42 53
MOBILE +31 (0) 6--16 94 04 90
FAX +31 (0) 24 – 350 21 54

E-MAIL sales@modiquestresearch.com
WEBSITE www.modiquestresearch.com

Edition 03.05.2007

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 (NaN_3).
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 (NaN_3), 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 compromise 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.

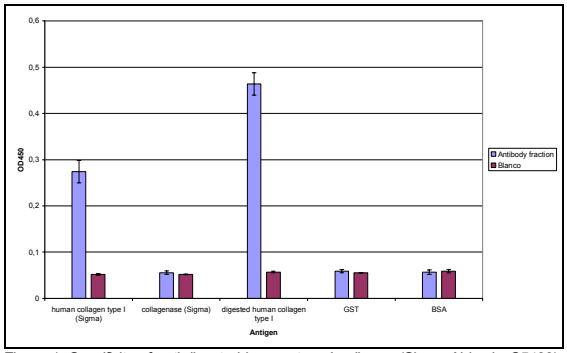

Figure 1: Specificity of anti-digested human type I collagen (Sigma-Aldrech, C5483), determined by ELISA. Antibody fraction (2mg/ml) 1800X diluted in MPBST. Antibody was tested on various recombinant protein substrates i.e. Human type I collagen (Sigma-Aldrich, C5483), GST (pGEX-2T) and BSA, 98% (Sigma).

RADE REGISTER Arnhem 09148575
V.A.T. NL 8140.81.058.B01
BANK ACCOUNT Postbank 5261629
IBAN NL90PSTB0005261629
SWIFT/BIC PSTBNL21

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

CAT No.
MQ3.101-100

Size
100 µg

ModiQuest RESEARCH

POSTAL ADDRESS Schoutstraat 58
6525 XV Nijmegen
The Netherlands

TELEPHONE +31 (0) 24 – 361 42 53
MOBILE +31 (0) 6--16 94 04 90
FAX +31 (0) 24 – 350 21 54

E-MAIL sales@modiquestresearch.com
WEBSITE www.modiquestresearch.com

Edition 03.05.2007

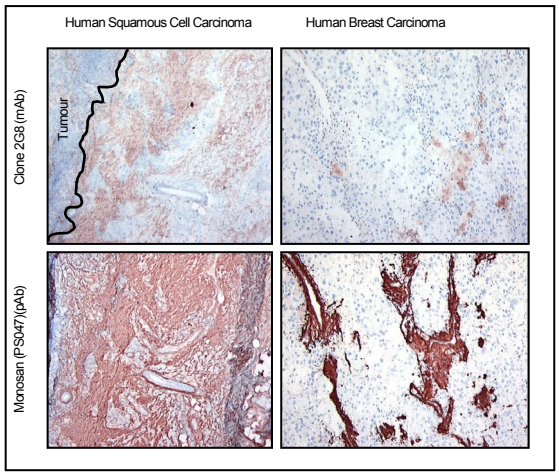

Figure 2: Frozen 4µm sections of human squamous cell carcinoma and human breast carcinoma where stained with anti-Collagenase Digested Human Type I collagen (2G8) (dilution 1:100). Goat anti-Mouse-HRP was used as secondary antibody and developed with 3-amino-9-ethylcarbazole (AEC) according to standard 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.

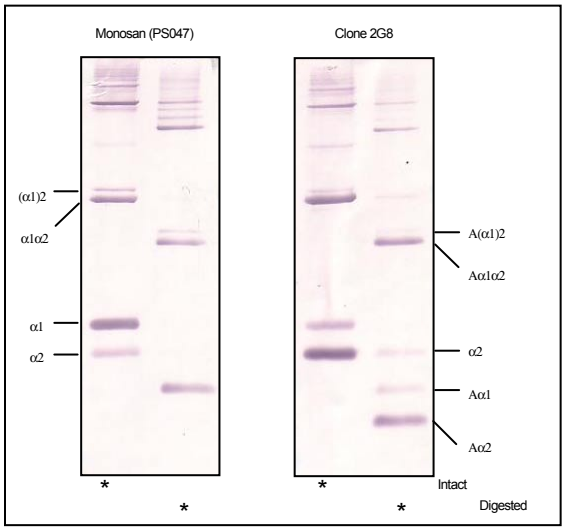

Figure 3: 1µg of intact or matrix metalloproteinase (MMP)-1 (Chemicon) digested human type I collagen was loaded in each lane of a 6% SDS-Page gel under non-reducing conditions which, after electrophoresis, was subjected to Western blotting. The blots where incubated with the Anti Collagenase Degraded 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 α1 and α2 chains are indicated. Capital A in front of the chains indicates digested forms.

References

1. Vuorio and Crombrugghe. 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.

RADE REGISTER Arnhem 09148575
V.A.T. NL 8140.81.058.B01
BANK ACCOUNT Postbank 5261629
IBAN NL90PSTB0005261629
SWIFT/BIC PSTBNL21