NYB•T2G1

Mouse Anti-Fibrin II β Chain (Bβ 15-42)

FORM: This monoclonal mouse anti-fibrin II β Chain (Bβ 15-42) antibody is supplied at 1mg/ml in 40mM Tris, 110mM NaCl, 0.1% NaN_3, (pH 7.5). The antibody has been purified from hybridoma medium using Protein G column chromotography. As supplied the antibody can be stored at +4°C or in small aliquots at -70°C and is stable for at least one year. An enzyme labeled form (horseradish peroxidase) of T2G1 is also available.

SPECIFICITY: NYB•T2G1 was produced using the NH_2-terminal CNBr fragment of human fibrin II called (T)N-DSK ([Aα 16-51, Bβ 15-118, γ 1-78]$_2$, M_r 52,000) as immumogen. The antibody cannot recognize fibrinogen but reacts fully with fibrin II (fibrin lacking both fibrinopeptide A and B), (T)N-DSK and fibrin II β-chain (Bβ 15-461) before and after cleavage with CNBr. **NYB•T2G1** also reacts with peptide Bβ 15-42, an early plasmin digest product of fibrin II.

APPLICATION: Fibrinogen is a large (M_r 340,000) blood glycoprotein. The molecule is a dimer composed of three pairs of non-identical polypeptide chains called Aα (M_r 48,000, 411 amino acid residues). The six fibrinogen chains are held together by 29 disulfide bonds, three of which (between corresponding residues at Aα28Cys, γ8Cys and γ9Cys) unite the two halves of the molecule at its NH_2-terminal end. Fibrinogen chains contain several prosthetic groups. Two carbohydrate clusters are present on both ,the Bβ and γ chains in each half of the molecule. Ester-bound phosphate and sulfate groups have been identified on Aα and Bβ chains of fibrinogen obtained from different species. In the electron microscope, fibrinogen appears as a rod like trinodular structure composed of a central E domain and the two identical distal D domains. The E domain is connected to the two D domains by coiled helices composed of all three fibrinogen chains.

The thrombin-catalyzed release of the two very small peptides, termed fibrinopeptide A (FPA, Aα 1-16) and fibrinopeptide B (FPB, Bβ 1-14), results in generation of fibrin monomer. Once formed, such fibrin molecules spontaneously begin to polymerize. The elongated, staggered fibrin polymer, often referred to as the protofibrin, results from the interaction of complementary binding sites on adjacent monomer molecules. In the terminal stage of blood clotting, fibrin is stabilized by introduction of ε-(γ-glytamyl)lysine cross-links between γ and α chains in adjacent fibrin monomers. This reaction is mediated by the plasma transglutaminase FXIIIa and the first cross-links introduced are between residues γ398Gln and γ406Lys in the COOH-terminal portions of the γ chains of adjacent fibrin monomers.

Proteolytic cleavage of fibrinogen with plasmin results in a number of well-defined fragments. Heterogeneity of some of these fragments is more restricted when digestion buffers contain calcium ions in the range 2-10mM. Under such conditions, fibrinogen is progressively cleaved to transient degradation products called Fragment X (Fg-X, M_r 150,000 to 170,000) as well as terminal core products called Fragment D (Fg-D, M_r 93,000) and Fragment E (Fg-E, M_r 50,000). The generation of Fg-X is accompanied by cleavage of heat stable fragments from the COOH-terminal region of fibrin(ogen) Aα-chain and the NH_2-terminal region of fibrinogen Bβ-chain (Bβ1-42) or fibrin II β-chain (Bβ 15-42). ,A very similar pattern of degradation is obtained when unstabilized fibrin is used as substrate. When FXIIIa cross-linked fibrin is cleaved with plasmin, the terminal core fragments differ from those identified above. Instead of Fg-D, plasmin-cleaved cross-linked fibrin yields a dimerized Fragment D species (Fg-DD or D-dimer, M_r 186,000). Depending on the extent of proteolysis, several different Fg-E species have been identified in plasmin digests of cross-linked fibrin.

CLINICAL SIGNIFICANCE: Since **NYB•T2G1** fails to react with fibrinogen, the antibody is a useful probe for: *in vivo* thrombus detection; identification of soluble fibrin monomer in plasma; distribution of fibrin II in tissues; screening early events in thrombus dissolution.

USAGE: NYB•T2G1 has now been shown to be effective blood clot imaging agent. This antibody is also useful in monitoring the efficacy of fibrinolytic therapy and has been shown to be an important tool in immunohistochemistry for detection of fibrin II in tissues. **NYB•T2G1,** cross-reactive with fibrin II (3 chains from several other species (monkey and dog), can also be used for western blotting (1:500 or 1:1000 starting).

ELISA: Need to titrate as it depends on antigen used. This antibody works on frozen and paraffin sections. Poor staining achieved with formalin or Glutaraldehyde fixatives (need to do target unmasking). Use with non-highly cross-linking fixative acceptable.

For research only. Not for human or diagnostic use.

REFERENCES:

Kudryk, B., Rohoza, A., Ahadi, M., Chin, J. and Wiebe, M.E. Specificity of a Monoclonal Antibody for the NH_2-Terminal Region of Fibrin: *Molec.* Immun. 21: 89-94, 1984.

Rosebrough, S.F., Grossman, Z.D., McAfee, J.G., Kudryk, B.J., , Subramanian, G., Ritter- C.A., Witanowski, L.S., Tillapaugh-Fay, G. and Urrutia, E. Aged Venous Thrombi: Radioimmunoimaging with Fibrin-Specific Monoclonal Antibody. *Radiology* 162: 575-577, 1987.

Bini, A., Fenoglio, J.J., Jr., Mesa-Tejada, R., Kudryk, B. and Kaplan, K.L. Identification and Distribution of Fibrinogen, Fibrin and Fibrin(ogen) Degradation Products in Atherosclerosis by Monoclonal Antibodies. *Arteriosclerosis* 9(1): 109-121, 1989

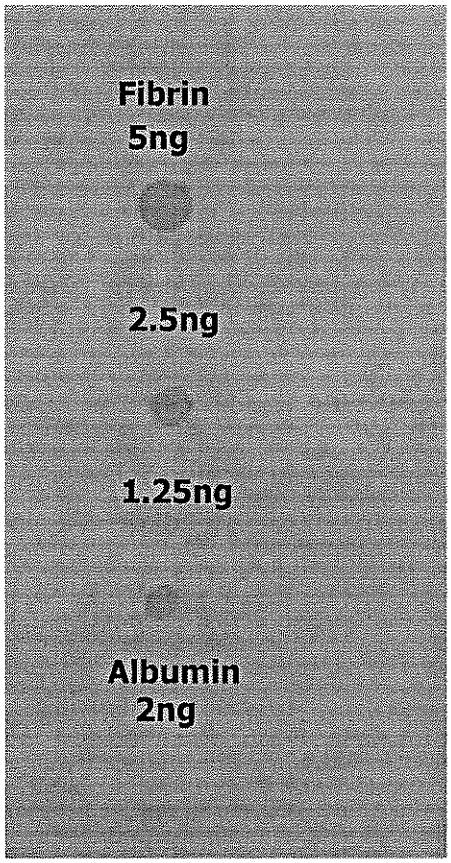
Kudryk, B.J., Grossman, Z.D., McAfee, J.G. and Rosebrough, S.F. Monoclonal Antibodies as Probes for Fibrin(ogen) Proteolysis. In: Monoclonal Antibodies in Immunoscintigraphy (J-F. Chatal, ed.) CRC Press, Boca Raton, Florida, pp 365-398, 1989.

Rosebrough, S.F., McAfee, J.G., Grossman, Z.D., Kudryk, B.J., Ritter-Hrncirik, C.A., Witanowski, L.S., Maley, B.L., Bertrand, E.A.;. and Gagne, G.M. Thrombus Imaging: A Comparison of Radiolabeled GC4 and T2G1s Fibrin-Specific Monoclonal Antibodies. j. Nucl. *Med. 31:* 1048-1054, 1990.

Procyk, R., Kudryk, B., Callender, S. and Blomback, B. Accessibility of Epitopes on Fibrin Clots and Fibrinogen *Gels.Blood 77*(7): 1469-1475, 1991.

Procyk, R., Medved', L., Engelke, K.J., Kudryk, B., and Blomback, B. Nonclottable Fibrin Obtained from Partially Reduced Fibrinogen: Characterization and Tissue Plasminogen Activator Stimulation. *Biochemistry* 31(8) 2273-2278, 1992.

Dot Blot Using NYB•T2G1



1µl dots of Human Fibrin resuspended in 500mM NaOH were applied to nitrocellulose and allowed to dry.

The membrane was blocked in 3% Non-Fat Dry Milk in Phosphate Buffered Saline + 0.05% Tween (PBS-T) for 30 min.

NYBT2G1 as the primary antibody, 1:2500 in blocking solution.
Incubation in primary antibody 30 min.

Goat anti-mouse secondary antibody conjugated to alkaline phosphatase (ALP), 1:10000 in blocking solution.
Incubation in secondary antibody 30min.

SDS-PAGE NYB●T2G1

10% Gel, Tris-HEPES-SDS buffer pH 8
Lane1: Hybridoma medium pre-protein G column (10µl)
Lane2: Protein Markers
Lane3: NYBT2G1 after Protein G column purification (10µl)
All samples were reduced