Anti-Human Fibrinogen Monoclonal Antibody - Ascites

CL1302A
LOT: B200

DESCRIPTION:
Fibrinogen is a large protein of 340,000 Da which is composed of three pairs of non-identical chains: \( \alpha \beta \beta \), and \( \gamma \) which are bound together by a series of disulfide bonds, and having a molecular mass of 66,062, 54,358 and 48,529 Da respectively. The overall structure may be described as \( (\alpha \beta \beta, \gamma)^2 \). Fibrinogen is found primarily in the plasma where it plays an essential and central role in the clotting cascade. During the process of coagulation, it is the cleavage of fibrinopeptide A and fibrinopeptide B from the \( \alpha \) and \( \beta \) chains by thrombin, which converts fibrinogen to fibrin and starts its polymerization. Fibrinogen is also present in the \( \alpha \) granules of platelets and has been found to be essential for platelet aggregation in response to ADP, collagen and thrombin.1

Cedarlane's anti-fibrinogen monoclonal antibody specifically recognizes fibrinogen and is able to inhibit the activity of this protein in coagulation and platelet aggregation in vitro.

Thus, this antibody either recognizes or is able to sterically hinder specific sites on the fibrinogen molecule which are important for its role in both coagulation and platelet aggregation. Since the antibody clearly affects these two unique properties of fibrinogen, it may be useful for the further delineation of these processes.

PRESENTATION: 0.5 ml, lyophilized

STORAGE AND RECONSTITUTION: Store at -20°C or below before reconstitution. Reconstitute with 0.5 ml of distilled water. Aliquot and freeze the unused portion in volumes appropriate for single use, as repeated freezing and thawing may reduce antibody activity.

STERILITY: This reagent is not sold as sterile, but can be sterilized by filtration if necessary. To minimize loss of volume during filtration, dilute to the final working concentration in the appropriate medium before filtration and filter through a 0.22µ Millipore filter (or equivalent).

SPECIFICATIONS:
LOT: B200
CLONE: 2C2-G7

Hybridoma Production: Immunizing Strain: BALB/c
Immunogen: Fibrinogen purified from a preparation of "Standard Antihaemophilic Factor" (AHF)1
Fusion: Spleen cells from immunized recipient were fused with myeloma P3-NS1-1-Ag4 (NS-1)

Specificity: Human Fibrinogen
Ig Class: Mouse IgG1, k light chain
Presentation: Ascites fluid

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Activity: This antibody has been assayed by an enzyme-linked immunosorbant assay (ELISA). Human fibrinogen was bound to the wells of a microtitre plate and sequential dilutions of CL1302A were added to the wells. After incubation and washing, alkaline phosphatase conjugated anti-mouse IgG was added to the wells. Colour reaction was developed and scored as intense (3+) to none (-). The titre is defined as the maximum dilution yielding a (3+) reaction.

End-Point Titre: >1:50,000 in an ELISA assay.

ANALYSIS BY SDS-PAGE AND WESTERN BLOTTING
Following electrophoretic separation on a SDS-PAGE gel, resultant subunit polypeptides were blotted to a nitrocellulose membrane and then incubated with anti-fibrinogen (CL1302A) (primary antibody) at a dilution of 1:40. Membrane was then washed and incubated with a secondary antibody (Goat Anti-Mouse IgG (H+L) HRPO conjugate (Human Absorbed) and antigen was detected visually by addition of HRP substrate solution.

Three bands were detected ranging from 48,000 Da to 66,000 Da. Each of these values correspond to a known molecular weight for the subunit polypeptides of fibrinogen.

This antibody is specific for human fibrinogen. No cross-reactivity with other human serum proteins as tested in ELISA. No cross-reactivity with mouse Ig or with normal serum from other animal species.

Additional Testing performed on 2C2-G7 clone:

Solid phase RIA
2C2-G7 was found to have a supernatant titre of 1:800 when tested by solid phase RIA against fibrinogen.

Note: Since fibrinogen is known to be involved in both coagulation and collagen-induced platelet aggregation, the effect on these functions was tested.

EFFECT OF ANTI-FIBRINOGEN MONOCLONAL ANTIBODY ON ACTIVATED PARTIAL THROMBOPLASTIN TIMES:
Activated partial thromboplastin times (APTT'S) give a measure of the efficiency of the intrinsic coagulation pathway. Since fibrinogen is involved in this pathway, the effect of the anti-fibrinogen monoclonal antibody on the APTT of normal plasma was tested. 2C2-G7 was found to inhibit APTT by 30% indicating that the antibody recognizes a determinant at or near a site on the fibrinogen molecule which is important for the involvement of the molecule in the coagulation cascade.

However, since the APTT assay measures the efficiency of the intrinsic coagulation pathway as a whole, it is not a very specific indicator of fibrinogen in coagulation.

For this reason, the thrombin time of normal plasma in the presence of the anti-fibrinogen was also tested.

EFFECT OF ANTI-FIBRINOGEN MONOCLONAL ANTIBODY ON THROMBIN TIMES:
Thrombin times give a measure of the rate at which fibrinogen is incorporated into stable fibrin polymers.

The antibody was tested for inhibitory effects on the thrombin time of normal plasma. Varying dilutions of normal plasma were tested to ensure that high plasma levels of fibrinogen were not simply swamping the antibodies. The results showed that inhibition of thrombin time did occur at only low concentrations of normal plasma.

A lack of inhibition was noted at 75%-100% normal plasma thus indicating that concentrations of plasma fibrinogen are able to successfully saturate the antigen binding sites on the antibody and abolish its effect.

(At 20% normal plasma, 2C2-G7 caused a 32% inhibition of thrombin times).

Therefore, this monoclonal antibody is able to inhibit the action of thrombin on fibrinogen or to destabilize the fibrin polymers to such an extent that inhibition of thrombin time occurs.

EFFECT OF ANTI-FIBRINOGEN ON COLLAGEN-INDUCED AGGREGATION OF WASHED PLATELETS:
The antibody was tested for inhibition of collagen-induced platelet aggregation in a washed platelet system to further specify the nature of the antibody-fibrinogen interaction.

At 20 µg / ml of collagen, 2C2-G7 inhibited the rate of platelet aggregation by 90%.

From these results, it would appear that the anti-fibrinogen monoclonal antibody recognizes determinants on the molecule which are directly involved in collagen induced platelet aggregation.

PROTEIN CONCENTRATION: 47.4 ± 2.6 mg /ml

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REFERENCES:
   Research Centre for Cancer and Transplantation  
   Dept. of Pathology, University of Melbourne - Unpublished

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