Anti-Human Factor VIII:vWF
Monoclonal Antibody

CL1303A - Ascites
CL1303AP – Purified

DESCRIPTION:
Factor VIII (FVIII) circulates in the plasma as a non-covalently bound complex of two antigenically and functionally distinct proteins including the FVIII procoagulant protein (VIII:C) and the Von Willebrand factor (vWF) protein. This FVIII complex is an essential component in the sequence of events which lead to blood coagulation and platelet function. The FVIII procoagulant protein (VIII:C) has been shown to correct the coagulation disorder of Haemophilia A, and the FVIII:vWF protein is able to correct the bleeding time abnormality associated with von Willebrand’s disease. The FVIII:vWF has been identified as a high molecular weight glycoprotein comprising a series of disulphide-bonded multimers ranging in molecular weight from approximately 800,000 da to over 12 x 10^6 da. It is synthesized by endothelial cells and megakaryocytes. The secretion of vWF by the vascular endothelium can be either in the circulation or abluminal, in which case the protein becomes part of the subendothelial matrix. The vWF of megakaryocytes is packaged in the α-granules of platelets for secretion at the time of platelet activation. vWF participates in hemostasis by mediating the adhesion of platelets to exposed subendothelium and promoting the formation of platelet thrombi at sites of vascular injury. Moreover, vWF forms a non-covalent complex with Factor VIII and prevents rapid removal of this procoagulant protein from circulation. vWF also likely promotes the localization of FVIII at the site of the developing clot. Thus, vWF interacts with cell receptors, insoluble components of the subendothelium, and a circulating protein. The multiplicity of binding functions justifies its’ definition as an “adhesive” protein.

Immunofluorescence and immunoperoxidase studies have shown FVIII:vWF to be localized to the endothelial cells of blood vessel walls. Thus, this antibody may be used for the histological identification of these cells on cryostat and paraffin sections. This antibody may also find further clinical application in the diagnosis of different forms of von Willebrand’s disease and in the affinity purification of FVIII:vWF for the treatment of patients.

This antibody may also be used to identify a variety of vascular tumours such as haemangiomas, hemangiosarcomas and Kaposis’s sarcoma, as FVIII:vWF is an endothelial cell marker in normal tissue as well as in malignantly transformed endothelial cells.

PRESENTATION:

Ascites (CL1303A): 0.5 ml, lyophilized
Purified (CL1303AP): 200 µg Purified IgG buffered in PBS and 0.02% NaN₃. (Purified from ascitic fluid via Protein G Chromatography). For maximum recovery of contents, spin down tube before use.

STORAGE AND RECONSTITUTION: Store ascites at -20°C or below before reconstitution. Reconstitute with 0.5 ml of distilled water. Store the purified format at 4°C. For long term storage aliquot and freeze the unused portion in volumes appropriate for single use, as repeated freezing and thawing may reduce antibody activity.

STERILITY:
These reagent are 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 0.22µm filter.
SPECIFICATIONS:
Clone: 2F2-A9
Hybridoma Production: Immunizing Strain: BALB/c
Fusion: Spleen cells from immunized recipient were fused with myeloma P3-NS1-1-Ag4 (NS-1).
Immunogen: Semi-purified human factor VIII:vWF.
Specificity: Human Factor VIII:vWF (von Willebrand’s Factor)
Ig Class: Mouse IgG1, κ light chain

RECOMMENDED METHOD FOR IMMUNOFLUORESCENCE STAINING OF HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS WITH CL1303A

PROTOCOL:
1. Endothelial cells derived from human umbilical veins are cultured on glass coverslips until confluency is reached.
2. Coverslips are washed several times with PBS (phosphate buffered saline) to remove traces of serum.
3. Cells are fixed by the addition of methanol at room temperature for 10 minutes.
4. Coverslips are washed three times with PBS and incubated with CL1303A (anti-FVIII:vWF) diluted 1:50 in 0.2% gelatin in PBS for 1 hour at room temperature.
5. Coverslips are washed three times with PBS (5 minutes each).
6. Goat anti-mouse IgG-FITC (*CC30201) was added, diluted 1:50 in 0.2% gelatin in PBS.
7. Incubate for 1 hour at room temperature in a moist chamber.
8. Wash coverslips three times with PBS (5 minutes each).
9. Coverslip is embedded in 10% glycerol in PBS and examined with a fluorescent microscope.

*CC30201 - Goat anti-mouse IgG (H+L) Rat absorbed Affinity Isolated Antibody - FITC conjugate is recommended for use in the detection of CL1303A in an indirect immunofluorescent assay.

RESULTS OF IMMUNOFLUORESCENCE ASSAY ON HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS

CL1303A is very well suited for the specific immunohistochemical demonstration of Factor VIII related antigen. (i.e. von Willebrand’s Factor).
Indirect Immunofluorescence showed a distinct granular type of staining present in the cytoplasm of the endothelial cells. In addition, a faint diffuse type of immunofluorescence around the nucleus was noted.

RECOMMENDED WORKING DILUTIONS:

Immunofluorescence and Immunoperoxidase staining: 1:50
Immunoperoxidase staining on Formalin-fixed, paraffin embedded tissue: 1:200.

ADDITIONAL TESTING COMPLETED ON THE 2F2-A9 HYBRIDOMA

Solid Phase Radioimmunoassay
This assay was performed for detection of the antibody and to determine antibody specificity. The results showed the VIII:vWF monoclonal antibody to be positive on VIII:vWF protein but negative on both fibrinogen and fibronectin.
Platelet Radioimmunoassay
This was a highly sensitive assay performed to detect all surface antigens on platelets. The results indicated that 2F2-A9 was non-reactive with platelets and thus that resting platelets (that is, unstimulated) do not express surface VIII:vWF.

von Willebrand’s Factor Assay
In past studies, the VIII:vWF protein has been shown to have ristocetin cofactor activity (vWF:RCo) which is the ability to promote platelet aggregation in the presence of the antibiotic ristocetin. In this assay, this activity was measured by the rate at which formalin-fixed platelets (FFP) agglutinated in the presence of normal plasma and ristocetin. The clone 2F2-A9 was tested for its ability to inhibit the functional activity of vWF:RCo activity. Results showed a 52% and 48% inhibition of VIII:vWF activity at 50% and 25% normal plasma, respectively. These results thus indicate that the monoclonal has the ability to affect sites on the VIII:vWF molecule that are of functional significance for vWF:RCo activity.

FACTOR VIII. PROCOAGULANT ASSAY

As mentioned previously, the FVIII complex is a non-convalently bound complex of 2 distinct proteins including the FVIII procoagulant protein (VIII:C) and the vWF protein. Therefore, in order to delineate the specificity of the antibody, 2F2-A9 was tested for any anti-VIII:C activity. The effect of the antibody directed against VIII:vWF on VIII:C activity was determined by incubating equal volumes of 10X concentrated hybridoma supernatant with dilutions of normal plasma for 2 hours, and assaying for VIII:C activity at time zero and time 2 hours. The % residual activity of VIII:C after 2 hours remained at 100%, thus the antibody showed no inhibition of activity implying that the antibody does not specifically react with the VIII:C protein.

* For optimal results in various applications, it is recommended that each investigator determine dilutions appropriate for individual use.
REFERENCES: