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monoclonal antibody anti-human Tissue Factor (CD142) IgG

REF 4509

Description

Tissue Factor (TF) is a 47,000 D transmembrane cell surface glycoprotein known for its role in initiating coagulation. It is comprised of three domains: an extracellular domain (aa 1-219), a hydrophilic spanning domain (aa 220-242) and a cytoplasmic tail (aa 243-263). Released into the blood stream following disruption of the endothelium, tissue factor functions as a receptor and cofactor for factors VII and VIIa. Contact between TF and blood is sufficient to initiate the extrinsic pathway of coagulation. This initiation requires the participation of a series of molecules; factor VII, factor X or factor IX, charged phospholipids and calcium ions, and their ability to complex with tissue factor. The TF/FVIIa complex efficiently activates both factor X and factor IX, thus initiating both the intrinsic and extrinsic coagulation pathways.

REF 4509 is directed against an epitope within aa 1-25, the extracellular domain of human tissue factor. The monoclonal reacts with and neutralizes the purified apoprotein of human tissue factor, native human brain and placental thromboplastin, recognize tissue factor on the surface of tumor cells and LPS stimulated monocytes. At very high concentrations, REF4509 will inhibit rabbit TF.

Preparation

REF 4509 is a murine IgG_1 monoclonal antibody secreted by hybridomas generated by the fusion of Balb/c spleen cells with the P3Ag8.653.1 myeloma cell line. The antibody is purified from cell culture via Protein G affinity chromatography. Purified apoprotein of human tissue factor, M_r = 47,000 D, was used as the immunizing antigen.

Presentation

Clear glass, screw-capped vials containing 0.5 mg of purified IgG_1, lyophilized from a 0.5 mg/mL solution in 0.15M PBS, pH 6.8 with 100 mM mannitol added as an excipient.

Reconstitution

Add 1.0 mL of filtered deionized or sterile water to the vial to generate a 0.5 mg/mL stock solution.

Storage

Store lyophilized antibody at 2°-8°C. Aliquot and store reconstituted antibody at –20°C or colder.

Related Products

Tissue Factor, rec human REF 4500 & relipidated TF REF 4500L/B

Goat polyclonal antibody against human TF, REF 4501

Mouse MAb against human TF, REF 4503

IMUBIND® Tissue Factor ELISA, REF 845

ACTICHROME® TF REF 846

Applications

A. Immunohistochemistry – REF 4509 successfully stains both cryo- and formalin-fixed, paraffin-embedded human sections of carotid and atherosclerotic arteries,[1,2] cardiac tissue,[3] pancreatic tissue[4] and malignant breast tumor tissue.[7] Concentrations ranging from 1-10 µg/mL using incubations of 1 hour at 18°-25°C or overnight at 4°C have been used.

B. Inhibition of TF Procoagulant Activity - At a 1:50 dilution, REF 4509 completely inhibited the generation of Factor Xa activity in a single-stage chromogenic assay involving LPS stimulated HUVEC.[5] It also inhibited the procoagulant effect of cross-linked human monocytic THP-1 cells in a single-stage clotting assay.[6]

C. Western Blot Analysis – REF 4509 is suitable for Western blot analysis,[8] detecting 50 ng of human tissue factor under reducing and non-reducing conditions at a working concentration of 5-10 µg/mL.

Bibliography

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3. Yen, M. H., *et al*. Increased Tissue Factor Expression Predicts Development of Cardiac Allograft Vasculopathy. *Circulation* 2002, **106**: 1379-1383.
4. Beuneu, C., *et al*. Human Pancreatic Duct Cells Exert Tissue Factor-Dependent Procoagulant Activity, Relevance to Islet Transplantation. *Diabetes* 2004, **53**: 1407-1411.
5. Pouplard, C., *et al*. A Simplified and Low-Cost One-Stage Chromogenic Assay for Tissue Factor Dependent Procoagulant Activity of Endothelial Cells. *Thrombosis Research* 1995, **80**: 527-534.
6. McGilvray, I. D., *et al*. VLA-4 Integrin Cross-linking on Human Monocytic THP-1 Cells Induces Tissue Factor Expression by a Mechanism Involving Mitogen-activated Protein Kinase. *Journal of Biological Chemistry* 1997, **272**: 10287-10294.
7. Contrino, J., *et al*. In Situ detection of tissue factor in vascular endothelial cells: Correlation with the malignant phenotype of human breast disease. *Nature Medicine* 1996, **2**: 209-215.
8. Ritis, K., *et al*. A Novel C5a Receptor-Tissue Factor Cross-Talk in Neutrophils Links Innate Immunity to Coagulation Pathways. *The Journal of Immunology* 2006, **177**: 4794-4802.

Warnings and Precaustions

For Research Use Only.

This product contains source material of a biological origin and should be handled using Universal Precautions.

Immunohistochemical Staining of Tissue Factor in Formalin-Fixed Paraffin Embedded Tissue Sections Using REF 4509

A) Dewaxing

1. Dewax a 3-5 µm tissue section on a poly-L-lysine coated slide with xylene for 6-10 minutes.

B) Rehydration

2. Rehydrate the tissue section with successive dilutions of ethanol (EtOH) at room temperature as follows:

100% EtOH	4 minutes
95% EtOH	2 minutes
75% EtOH	2 minutes
50% EtOH	2 minutes

C) Blocking

3. Block endogenous peroxidase activity by immersing the tissue section in a 0.3% H_2O_2 in methanol solution for 15 minutes at 4°C.
4. Block the tissue section for 1 hour at room temperature with a solution of 10% normal goat serum in 0.15M PBS, pH 7.4. Wash the section tissue 3 times with 0.15M PBS. Blot the section to remove excess fluid.

D) Staining

5. Apply MAb REF 4509 at a 1:50 dilution (10 µg/mL) in Assay Solution and incubate overnight (16 hours) at 4°C.
6. Wash the section 3 times with Assay Solution, 5 minutes for each wash.
7. Apply peroxidase labeled goat anti-mouse at a 1:100 dilution in Assay Solution and incubate for 1 hour at room temperature.
8. Wash the section 3 times with Assay Solution, 5 minutes for each wash.
9. Apply Substrate Solution and incubate for 30 minutes at room temperature.
10. Wash the section 3 times with Assay Solution, 5 minutes for each wash.
11. Counterstain the section with Mayer's hematoxilyn for 10 minutes then rinse the extensively with distilled water.
12. Remove excess fluid, mount sections and apply cover slip. Allow the section to dry for 1 hour. Positive tissue factor staining is visualized by brownish coloring.

Assay Solution:	0.15M PBS, 1% BSA, 0.3% Tween 20, pH 7.4
Blocking Solution:	10% Normal Goat Serum in 0.1%M PBS, pH 7.4
Substrate Solution:	3-amino-9-ethylcarbazole (AEC) in 0.1M Sodium Acetate, pH 5.2. Dissolve 4 mg AEC in 1 mL of N, N-dimethylformamide (0.4% solution, stable at 4°C for approximately 1 week). Add to 14 mL of acetate buffer. Add 0.15 mL of 3% H_2O_2 immediately prior to use.

Procedural Notes

1. Optional - Increased sensitivity may be obtained by performing a microwave treatment of the tissue section after dewaxing and rehydrating. Immerse the section in 0.01M sodium citrate buffer, pH 6.0 and microwave (1500W) two (2) times for 5 minutes each.
2. Do not include sodium azide as an anti-bacterial agent in the preparation of any solution used with peroxidase staining procedures. Azide inhibits peroxidase activity.
3. Do not let tissue sections dry at any time during paraffin removal or staining procedure.
4. Goat sera for blocking and goat secondary antibody are suggested. Any preimmune sera with corresponding species second antibody may be used.

Definition of Symbols


Consult instructions for use


Refer to SDS

Lot Number

Catalog Number


Manufacturer


Temperature Limitation


Expiration Date