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FITC-conjugated anti-human Tissue Factor

REF 4507CJ Lot No. YYMMDD

Description

Tissue Factor (TF, CD142) is a 45 kDa 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.

Monocytes and macrophages stimulated by endotoxins, cytokines and lectins, upregulate their level of tissue factor and an increase in procoagulant activity (PCA) is found. TF is also found in lung, brain, trophoblastic microvilli, placenta and some neoplastic tissues (e.g. benign breast carcinomas and melanomas).

REF 4507, clone VIC7, recognizes an epitope within amino acids 203-214 of human Tissue Factor.[1] The antibody neutralizes brain and placental thromboplastin and is useful in flow cytometry (direct immunofluorescence).[1,2]

Preparation

REF 4507CJ, FITC conjugated clone VIC7, is a mouse IgG_1 monoclonal antibody purified from cell culture via Protein G affinity chromatography. Free FITC is removed by gel filtration. Purified tissue factor apoprotein was the immunizing antigen.

Fluorescein/Protein Molar Ratio

X.X : 1

Presentation

50 µg of FITC conjugated purified IgG lyophilized from 500 µL of a buffer of 0.15M Phosphate Buffered Saline, 1% BSA, 0.01% gentamicin, pH 7.4.

Reconstitution

Add 500 µL of filtered deionized or distilled water to dissolve the antibody and generate a 100 µg/mL stock solution. Further dilutions should be made with 0.15 M PBS, 1% BSA, pH 7.4, if necessary.

Storage

Store lyophilized product ***in the dark*** at 2°-8°C. Reconstituted antibody may be stored ***in the dark*** at -20°C or colder for up to 1 month.

Applications

A. Flow Cytometry (See protocols on the reverse side)

At a concentration of 50-100 µg/mL, REF 4507CJ may be used for analyzing tissue factor expression of a wide variety of cell types including monocytes and HUVEC.[2]

NOTE: REF 4507CJ has reported to successfully analyze tissue factor expression of platelets.

B. Direct Immunofluorescence

REF 4507CJ has been used for direct immunofluorescence and has shown a strong reactivity for tissue factor in frozen tissue sections of both the kidney (glomerular epithelia) and the epidermis (upper cell layer).[2]

References

1. Magdolen, V., *et al*. *Biol Chem* 1998, **379:** 157-165.
2. Morrissey, J. H., et al. CD142 (tissue factor) Workshop Panel report. In: *Leucocyte Typing VI: white cell differentiation antigens.* Garland Publishing, Inc., New York, 1997, 742-746.
3. Amirkhosravi, A., *et al*. *Thrombosis and Haemostasis* 1996, **75**: 87-95.
4. Holmes, V. A., *et al*. *Thrombosis and Haemostasis* 2002, **87**: 953-958.
5. Luther, T., Flossel, C., Hietschhold, V., Koslowski, R. and Müller M. *Blut* 1990, **61:** 375-378.
6. Müller, M., Albrecht, S. and Golfert, F. *Experimental Cell Research* 1999, **248:** 136-147.

Related Products

IMUBIND® Tissue Factor ELISA (REF 845), ACTICHROME® TF Activity (REF 846), ACTICHROME® TFPI Activity (REF 848). Polyclonal and monoclonal anti-human TF antibodies (REF 4501, 4502, 4503, 4509, 4507CJ). Polyclonal antibody anti-mouse tissue factor (REF 4515).

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Flow Cytometry Protocols

A. Whole Blood Assay for Monocyte Tissue Factor Expression (Protocol used by Ref. 3)

1. Citrated whole blood (collected with 3.2% tetrasodium citrate) is stimulated with 10 µg/mL LPS (lipopolysaccharide) for 1 hour at 37°C. A sample of blood incubated without LPS may serve as a baseline control.
2. Add 100 µL of whole blood (LPS stimulated and baseline control from step 1) to a polypropylene tube containing 10 µL of anti-human CD14^{RD1} and 10 µL of REF 4507CJ. As a negative isotype control, add 10 µL of mouse IgG$_1$-FITC in place of 4507CJ.
3. Immediately place all tubes on ice for 30 minutes in the dark.
4. The blood in each tube is fixed by adding 250 µL of a mixture of formaldehyde (9.25%) and methanol (3.25%) and vortex gently for 15 seconds. After exactly 60 seconds of fixation, add 4 mL of Tyrode's Buffer and incubate for 30 minutes at room temperature in the dark. This fixation method will result in the complete lysis of the red blood cells.
5. Wash the samples by centrifuging for 3 minutes at 1000 rpm, decanting the supernatant and resuspending the pellet in 4 mL of Tyrode's Buffer. Perform this wash step two times.
6. Resuspend the pellet in 4 mL of Tyrode's Buffer.
7. The monocyte population is identified by gating the CD14 positive cells.
8. Negative and positive determinations are made by gating 2% background staining on the mouse IgG$_1$ isotype control.

Negative Isotype Control:

Mouse IgG$_1$-FITC, BD Biosciences, Cat. No. 340755, for US Market.
Mouse IgG$_1$-FITC, BD Biosciences, Cat. No. 3458155, for European Market.

Anti-CD14^{RD1}: Beckman Coulter (Cat. No. 6603262).

Tyrode's Buffer:

137 mM NaCl, 2.8 mM KCl, 1 mM $MgCl_2$, 12 mM $NaHCO_3$, 0.4 mM Na_2HPO_4, 0.35% BSA, 10 mM HEPES, 5.5 mM Dextrose, pH 7.4.

B. Monocyte Tissue Factor Expression of Isolated Peripheral Blood Mononuclear Cells (PBMC) (Protocol modified from Ref. 4).

1. Peripheral Blood Mononuclear Cells (PBMC) may be isolated from citrated whole blood (collected with 3.2% trisodium citrate) via sedimentation on a Ficoll-Paque™ gradient (Amersham Biosciences). Resuspend cells to a density of 10^6 cell/mL in RPMI supplemented with 5% fetal calf sera in polypropylene tubes. Alternatively, cells may be isolated via a buoyant density centrifugation followed by affinity purification with an anti-CD14 antibody column.
2. Cells are stimulated with 10 µg/mL of LPS for 4 hours at 37°C. Cells incubated without LPS may serve as a baseline control for tissue factor expression.
3. After stimulation, the cells are washed twice with cold PBS and fixed in 2 mL of cold 1% formaldehyde in PBS for 30 minutes in the dark. Then the cells are washed an additional two times with PBS.
4. 50 µL of fixed cells at 10^6 cell/mL (LPS stimulated and baseline samples) are added to a polypropylene tube containing 10 µL of REF 4507CJ and 10 µL of anti-human CD14-PE. As a negative isotype control, add 10 µL of mouse IgG$_1$-FITC instead of 4507CJ.
5. Immediately place all tubes on ice for 30 minutes in the dark.
6. Wash the samples by centrifuging for 3 minutes at 1000 rpm, decanting the supernatant and resuspending the pellet in 4 mL of PBS. Perform this wash step two times.
7. Resuspend the pellet in 4 mL of PBS.
8. The monocyte population is identified by gating the CD14 positive cells.

The effects of LPS stimulation and stimulation time were examined using the above protocol and the following results obtained.

Stimulation of MBC For 4 Hours	With LPS at 37°C	Without LPS at 37°C	Without LPS at 4°C
Percentage of TF Positive Monocytes	64%	13%	1%

Stimulation of MBC	0 hours	2 hours	6 hours	24 hours	48 hours
Percentage of TF Positive Monocytes	3%	14%	85%	55%	22%

Negative Isotype Control:

Mouse IgG$_1$-FITC, BD Biosciences, Cat. No. 340755, for US Market.
Mouse IgG$_1$-FITC, BD Biosciences, Cat. No. 3458155, for European Market.

Anti-CD14^{RD1}: Beckman Coulter (Cat. No. 6603262).