

american diagnostica inc.

## FITC-conjugated anti-human Tissue Factor

Product No. 4508CJ

Lot No. 080320

### 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 resulting in an increase in procoagulant activity (PCA). TF is also found in lung, brain, trophoblastic microvilli, placenta and some neoplastic tissues (e.g. benign breast carcinomas and melanomas).

No. 4508, clone VD8, recognizes an epitope within amino acids 1-25 of human Tissue Factor.<sup>1</sup> The antibody neutralizes TF procoagulant activity most likely by interfering with FVII binding to TF.<sup>1,2</sup>

### Preparation

No. 4508CJ, FITC conjugated clone VD8, is a mouse IgG<sub>1</sub> 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

3.2 : 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, 4508CJ may be used for analyzing tissue factor expression of a wide variety of cell types including monocytes,<sup>2,5</sup> fibroblasts,<sup>7</sup> smooth muscle cells,<sup>8</sup> melanoma cells,<sup>9</sup> microparticles,<sup>10</sup> lymphoblastoids,<sup>11</sup> breast cancer cells<sup>12</sup> and pancreatic duct cells.<sup>13</sup>

**NOTE:** Experiments using No. 4508CJ to analyze tissue factor expression of platelets have reported weak and inconsistent results questioning the ability of No. 4508 to recognize platelet TF. This may be in part due to insufficient platelet activation.

#### B. Direct Immunofluorescence

No. 4508CJ has been used for direct immunofluorescence and has shown a strong reactivity for tissue factor in frozen and paraffin embedded tissue sections of both the kidney (glomerular epithelia) and the epidermis (upper cell layer).<sup>2,6</sup>

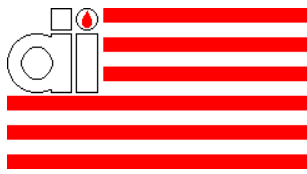
### References

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### Related Products

IMUBIND® Tissue Factor ELISA (No. 845), IMUBIND® Total TFPI ELISA (No. 849), ACTICHROME® TF Activity (No. 846), ACTICHROME® TFPI Activity (No. 848). Polyclonal and monoclonal anti-human TF antibodies (Nos. 4501, 4502, 4503, 4508, 4509, 4507CJ) and anti-human TFPI antibodies (Nos. 4901, 4904). Antibodies against rabbit tissue factor (Nos. 4510, 4511, 4513), mouse tissue factor (No. 4515) and mouse TFPI (No. 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<sup>RD1</sup> and 10 µL of No. 4508CJ. As a negative isotype control, add 10 µL of mouse IgG<sub>1</sub>-FITC in place of 4508CJ.
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 vortexing 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<sub>1</sub> isotype control.

### Negative Isotype Control:

Mouse IgG<sub>1</sub>-FITC, BD Biosciences, Cat. No. 340755, for US Market.

Mouse IgG<sub>1</sub>-FITC, BD Biosciences, Cat. No. 3458155, for European Market.

Anti-CD14<sup>RD1</sup>: Beckman Coulter (Cat. No. 6603262).

### Tyrode's Buffer:

137 mM NaCl, 2.8 mM KCl, 1 mM MgCl<sub>2</sub>, 12 mM NaHCO<sub>3</sub>, 0.4 mM Na<sub>2</sub>HPO<sub>4</sub>, 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<sup>6</sup> 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<sup>6</sup> cell/mL (LPS stimulated and baseline samples) are added to a polypropylene tube containing 10 µL of No. 4508CJ and 10 µL of anti-human CD14-PE. As a negative isotype control, add 10 µL of mouse IgG<sub>1</sub>-FITC instead of 4508CJ.
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 PMBC 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 PMBC	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<sub>1</sub>-FITC, BD Biosciences, Cat. No. 340755, for US Market.

Mouse IgG<sub>1</sub>-FITC, BD Biosciences, Cat. No. 3458155, for European Market.

Anti-CD14<sup>RD1</sup>: Beckman Coulter (Cat. No. 6603262).

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