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polyclonal antibodies against human tissue factor (CD 142) Product Nos. 4501 and 4502

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.

These high titer antibodies react with purified apoprotein of tissue factor, neutralizing lipidated tissue factor along with native tissue factor (Thromboplastin) derived from human brain as well as other sources of natural tissue factor (i.e. placenta acetone powder, tumor cells, LPS-stimulated monocytes).

No. 4501 partially neutralizes rabbit thromboplastin and recognizes rabbit recombinant tissue factor.

No. 45021 has been shown to inhibit rat Tissue Factor in a clotting assay (see Reference 2 below) and reacts with rat Tissue Factor in Western blots.

Preparation

Nos. 4501 and 4502 are polyclonal antibodies raised in goat and rabbit sera, respectively. Purified apoprotein of human tissue factor, $M_r = 47,000$ D, was used as the immunizing antigen. The antibodies are purified from the animal sera via Protein G (No. 4501) and Protein A (No. 4502) affinity chromatography.

Presentation

Clear glass, screw-capped vials containing 1.0 mg of purified IgG, lyophilized from a 1.0 mg/mL solution in 10 mM sodium phosphate, 140 mM sodium chloride, pH 7.4 with 100 mM mannitol added as an excipient.

Reconstitution

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

Storage

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

Applications

- A. **Western Blot Analysis:** Both antibodies are suitable for Western Blot procedures. A concentration of 1-2 µg/mL is recommended for the primary incubation (See Reference 1, 5).
- B. **Immunoprecipitation** – Both Nos. 4501 and No. 4502 may be useful for immunoprecipitation. A minimum 3:1 molar ratio of antibody:antigen is recommended.
- C. **Inhibition of TF Procoagulant Activity** – See the reverse page for details in inhibiting tissue factor procoagulant activity. No. 4501 inhibits tissue factor procoagulant activity, demonstrated in References 2 and 3.
- D. **Immunohistochemistry** – No. 4502 at 50 µg/mL successfully stained formalin-fixed paraffin embedded rat cardiac tissue when used with an HRP-labeled secondary antibody (Reference 4). No. 4501 at 33 µg/mL successfully stained frozen human cardiac tissue (Reference 5).

Bibliography

1. Contrino, J, *et al.* *Circulation* 1997, **96**(2): 605-613.
2. Watanabe, T., *et al.* Angiogenesis Induced by Tissue Factor in Vitro and in vivo. *Thrombosis Research* 1999, **96**: 183-189.
3. Lwaleed, Bashir. A., *et al.* Functional and structural properties of urinary tissue factor. *Nephrology Dialysis Transplantation* 1999, **14**: 588-596.
4. Nakanishi, Kuniaki, *et al.* Tissue Factor is associated with the nonbacterial thrombotic endocarditis induced by a hypobaric hypoxic environment in rats. *Virchows Arch* 1998, **433**: 375-379.
5. Yamani, Mohammad. H., *et al.* *Circulation* 2002, **105**: 1955-1961.

Related Products

Recombinant Tissue Factor, Product Nos. 4500 and 4500L (relipidated). Monoclonal antibodies against human Tissue Factor, Product Nos. 4508, 4509. IMUBIND® Tissue Factor ELISA, Product No. 845. ACTICHROME® TF Activity, Product No. 846. IMUBIND® Total TFPI ELISA, Product No. 849.

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Protocol for Neutralizing Brain Thromboplastin Coagulation Activity

A. Human Brain Thromboplastin:

- Place 50 µL of human brain thromboplastin (1:200 in TBS, acetone extract of human brain) into plastic tubes. Add 50 µL of Control (TBS) or purified anti-TF IgG at various dilutions in TBS (0.05M Tris-HCl, 0.1M NaCl, PH 7.6), Vortex
- Incubate for 30 minutes at 37° C
- Add 50 µL of normal human platelet-poor citrated plasma. Incubate the tube in a 37° C water bath for 1 minute
- Add 50 µL of prewarmed 30 mM CaCl₂ – Start timing the reaction
- Rock the tube continuously until the sample clots – Record the time in seconds
- Reference curve: Double logarithmic plotting of thromboplastin dilutions versus clotting times

Human Thromboplastin Dilution	1:50	1:100	1:200	1:400	1:800	1:1600	1:3200
Clotting Time, seconds	28	33	38	42	49	68	76
	29	33	37	43	50	69	77

Human Brain Thromboplastin at 1:200 dilution N° 4501 Stock Solution: 2 mg/mL
 N° 4502 Stock Solution: 2 mg/mL

Neutralizing Antibody	Antibody Dilutions						
	Control (TBS)	Stock	1:20	1:100	1:1000	1:10,000	1:100,000
4501	Clotting Time, seconds						
	38	115	110	94	61	45	38
Irrelevant Goat IgG	37	112	110	93	64	44	41
	38	38	37	38	38	37	37
4502	37	39	38	39	39	38	36
	38	109	107	64	42	39	39
Irrelevant Rabbit IgG	37	108	105	63	41	39	38
	38	38	37	38	38	37	38
	37	39	38	39	39	38	38

B. Rabbit Brain Thromboplastin:

Same as above substituting Rabbit Brain Thromboplastin at 1:200 dilution
 [N° 4501 Stock Solution: 2 mg/mL]

Neutralizing Antibody	Antibody Dilutions				
	Control (TBS)	Stock	1:20	1:100	1:1000
4501	36	109	85	54	36
	35	109	86	55	37
Irrelevant Goat IgG	35	38	37	38	38
	38	39	38	39	39