

IOTest[®] Anti-Thrombin Receptor-PE

PN IM2583 – 100 tests – 20 µL / test – Clone SPAN12

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

Thrombin is a coagulation protease produced at sites of vascular injury that activates platelets, endothelial cells, leukocytes and mesenchymal cells via cleavage of specific cell surface receptors known as proteinase activated receptors (PARs).

A functional thrombin receptor from human platelets has been cloned and sequenced (1). It is a 66-kDa, single polypeptide chain that belongs to the cell-surface G-protein-coupled receptor family, with seven transmembrane domains and an extracellular N terminus (1, 2).

The thrombin cleavage site is located in the N terminus between Arg41 and Ser42. Following cleavage by thrombin, activated receptors undergo desensitization and internalization but a fraction of them are recycled to the cell surface (3, 4).

SPAN 12 specifically reacts with the thrombin receptor, expressed on megakaryocytic cells, platelets and endothelial cells. A study, using this antibody, has shown that thrombin receptor is also expressed on a subpopulation of peripheral blood lymphocytes (about 30% of T cells and almost all CD56-positive natural killer cells) but not B cells, granulocytes and monocytes (5).

SPAN 12 recognizes the N-terminal peptide of thrombin receptor residues 35-46 (NATLDPR/SFLLR), where « / » indicates the putative site of cleavage by thrombin (4). SPAN12 is a « cleavage-sensitive » antibody since it reacts only with uncleaved thrombin receptors. In combination with WEDE15 antibody (PN IM2584), another monoclonal antibody reacting with both cleaved and uncleaved receptors, SPAN12 allows calculation of the proportion of intact receptors at the cell surface (6, 7).

SPAN12 inhibits thrombin-induced receptor activation and platelet aggregation at reduced thrombin concentrations (4).

The SPAN 12 monoclonal antibody was assigned to the Thrombin Receptor at the VIth International Workshop on Human Leukocyte Differentiation Antigens in Kobe, Japan (1996) (5).

REAGENT

IOTest Anti-Thrombin Receptor-PE

Conjugated Antibody

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Clone SPAN12

Isotype IgG1, mouse

Immunogen N-terminal peptide of thrombin receptor residues 35-46 (NATLDPRSFLLR).

Hybridoma SP2 x mouse Balb/c spleen cells

Source Ascites Fluid

Purification Protein A affinity chromatography

Conjugation R-phycoerythrin (PE) is conjugated at 0.5 – 1.5 moles of PE per mole of Ig

Excitation wavelength: 488 nm

Maximum emission wavelength: 575 nm

Main emission color: Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.

6. Avoid microbial contamination of reagents or incorrect results might occur.

7. Use good laboratory practices when handling this reagent.

APPLICATION

Studies of Thrombin-Receptor positive cells by flow cytometry.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored in the dark at 2 – 8°C. Do not freeze.

REAGENT PREPARATION

No reconstitution is necessary for this liquid form. Bring vial to 18 – 25°C prior to use.

PROCEDURE

This reagent is designed for flow cytometry.

Assay volume: 20 µL / 5 x 10⁵ cells / test or 10⁶ platelets or 100 µL of whole blood.

Notes:

Do not fix samples before staining.

SELECTED RESEARCH

REFERENCES

1. Vu, T.-K.H., Hung, D.T., Wheaton, V.I., Coughlin, S.R., "Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation", 1991, Cell, 64, 1057-1068.
2. Brass, L.F., Vassallo, R.R., Belmonte, E., Ahuja, M., Cichowski, K., Hoxie, J.A., "Structure and function of the human platelet thrombin receptor", 1992, J. Biol. Chem., 267, 13795-13798.
3. Hoxie, J.A., Ahuja, M., Belmonte, E., Pizzaro, S., Parton, R., Brass, L.F., "Internalization and recycling of activated thrombin receptors", 1993, J. Biol. Chem., 268, 13756-13763.
4. Brass, L.F., Pizzaro, S., Ahuja, M., Belmonte, E., Blanchard, N., Stadel, J.M., Hoxie, J.A., "Changes in the structure and function of the human

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- thrombin receptor during receptor activation, internalization and recycling", 1994, J. Biol. Chem., 269, 2943-2952.
- Fornelli, C., van Agthoven, A., "Non-lineage antigens clinical studies: expression and modulation of platelet-type thrombin receptors on lymphocytes, detected with anti-thrombin receptor monoclonal antibodies WEDE15 and SPAN12", 1997, in Leukocyte Typing VI, Kishimoto, T., ed., Garland Publishing Inc., pp. 618-621.
 - Woolkalis, M.J., Demelfi, T.M., Blanchard, N., Hoxie, J.A., Brass, L.F., "Regulation of thrombin receptors on human umbilical vein endothelial cells", 1995, J. Biol. Chem., 270, 9868-9875.
 - Molino, A.P., Abrams, C., Hoxie, J.A., Cerletti, C., M., Blanchard, N., Belmonte, E., Tarver Brass, L.F., "Proteolysis of the human platelet and endothelial cell thrombin receptor by neutrophil-derived cathepsin G", 1995, J. Biol. Chem., 270, 11168-11175.

PRODUCT AVAILABILITY

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For additional information in the USA,
call 800-526-7694.

Outside the USA, contact your local
Beckman Coulter representative.

www.beckmancoulter.com

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