

# Monoclonal Antibody Anti-Thrombin receptor

PN IM2085 – Purified – Freeze-dried – 0.2 mg – Clone WEDE15

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. 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 Arg<sup>41</sup> and Ser<sup>42</sup>. Following cleavage by thrombin, activated receptors undergo desensitization and internalization but a fraction of them are recycled to the cell surface (3, 4).

The WEDE15 monoclonal antibody (mAb) specifically reacts with the thrombin receptor, expressed on megakaryocytic cells, platelets and endothelial cells. The thrombin receptor is also expressed on a subpopulation of peripheral blood lymphocytes (about 30% of T cells and almost all the CD56-positive natural killer cells) but not on B cells, granulocytes and monocytes (5).

WEDE15 mAb recognizes specifically the N-terminal peptide of thrombin receptor residues 51-64 (KYEPFWEDEEKNES), (3). The WEDE15 mAb is a « cleavage-insensitive » antibody since it reacts with both cleaved and uncleaved receptors. In combination with SPAN12 mAb (reacting only with uncleaved receptor thrombin receptor), WEDE15 allows calculation of the proportion of intact receptors at the cell surface (4, 6, 7). The WEDE15 mAb has been studied at the Vth International Workshop on Human Leukocyte Differentiation Antigens in Kobe, Japan (1996) (5).

## REAGENT

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<b>Clone</b>	WEDE15
<b>Isotype</b>	IgG1
<b>Species</b>	Mouse
<b>Immunogen</b>	N-terminal peptide of thrombin receptor residues 51-64 (KYEPFWEDEEKNES).
<b>Hybridoma Source</b>	SP2 x Balb/c spleen cells
<b>Purification</b>	Ascites fluid
	Ion exchange or affinity chromatography

**Buffer** 1 mg/mL bovine serum albumin in phosphate-buffered saline

## APPLICATION

Studies of Thrombin receptor positive cells.  
Flow cytometry.

## STATEMENT OF WARNINGS

1. 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.
2. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
3. Do not use antibody beyond the expiration date on the label.
4. Avoid microbial contamination of reagents or incorrect results might occur.
5. Use good laboratory practices when handling this reagent.

## STORAGE CONDITIONS AND STABILITY

This freeze-dried form may be stored at 2 – 8°C until the expiration date stated on the vial label. No preservative has been added.

## REAGENT PREPARATION

Depending of usage, reconstitute with 1 mL of distilled water, with or without 0.1% sodium azide (w/v).

The reconstituted form including 0.1% sodium azide may be stored for up to one month at 2 – 8°C.

The reconstituted form without sodium azide can be stored at –20°C or less, until the expiration date stated on the vial label. In this case, aliquotting is recommended to avoid multiple freezing / thawing cycles.

## PROCEDURE

For each application, it is recommended to establish the right range of antibody dilutions to be used for the experiment.

## 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.

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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 thrombin receptor during receptor activation, internalization and recycling", 1994, J. Biol. Chem., 269, 2943-2952.
5. 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.
6. 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.
7. Molino, M., Blanchard, N., Belmonte, E., Tarver, A.P., Abrams, C., Hoxie, J.A., Cerletti, C., 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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