

Product Information

Monoclonal Anti-TRAIL, clone 75411

produced in mouse, purified immunoglobulin

Catalog Number **T3067**

Synonyms: Anti-Apo-2L, Anti-TNFSF10,
Anti-TNF-Related Apoptosis-Inducing Ligand

Product Description

Monoclonal Anti-TRAIL, Clone 75411 (mouse IgG₁) is produced in mouse using as immunogen purified recombinant human TRAIL extracellular domain expressed in mouse NSO cells.

Monoclonal Anti-TRAIL recognizes recombinant human TRAIL by immunohistochemistry and neutralization. This antibody was selected for its ability to neutralize the biological activity of human TRAIL.

TRAIL is a type II membrane protein from the TNF family.^{1,2} The human form of the protein is 281 amino acids in length; whereas, the murine form is 291 amino acids. The two forms share 65% identity. Recombinant human TRAIL monomer has a molecular mass of ~21 kDa. Due to glycosylation, recombinant human TRAIL migrates as a 24 kDa protein in SDS-PAGE.

Apoptosis or programmed cell death is induced in cells by a group of death domain-containing receptors. Binding of ligand to these receptors sends signals that activate members of the caspase family of proteases. The signals ultimately cause degradation of chromosomal DNA by activating DNase.

TRAIL initiates apoptosis of tumor cells by binding to either of its receptors, DR4 or DR5.³⁻⁶ These receptors consist of an extracellular TRAIL binding domain and a cytoplasmic "death domain". In addition, two decoy receptors for TRAIL have also been identified. These receptors, designated DcR1 and DcR2, lack the death domain. Binding of TRAIL to either of these receptors, therefore, does not transmit the death signal. Thus, these receptors represent a novel way of regulating cell sensitivity to a pro-apoptotic cytokine at the cell surface.^{7,8} TRAIL is expressed predominantly in spleen, lung, and prostate, but is also expressed in many other tissues.¹

Reagent

Lyophilized from 0.2 µm-filtered solution in phosphate buffered saline containing carbohydrates.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 µm filtered solution of phosphate buffered saline to produce a 0.5 mg/mL stock solution of antibody. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Storage/Stability

Prior to reconstitution, store at -20 °C. Reconstituted product may be stored at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at -20°C. Avoid repeated freezing and thawing. Do not store in a frost-free freezer.

Procedure

Monoclonal Anti-TRAIL neutralizes the bioactivity of human TRAIL in the presence of 12 ng/mL of cross-linked rhTRAIL, using mouse L929 cells. To measure the ability of this antibody to neutralize the biological activity of TRAIL, cross-linked recombinant human TRAIL is incubated with various concentrations of the antibody for 1 hour at 37 °C in a 96 well plate. Following this preincubation period, mouse L929 cells are added. The assay mixture in a total volume of 150 µL per well, containing antibody at concentrations of 0.01-100 ng/mL, cross-linked recombinant human TRAIL at 12 ng/mL, and actinomycin D at 1 µg/mL, are incubated at 37 °C for 24 hours in a humidified CO₂ incubator. Following incubation, the cells are fixed with 5% formaldehyde and then stained with a crystal violet solution containing 100 µL of 33% acetic acid. Absorbance at 540 nm is read on a microplate reader.

Product Profile

Immunohistochemistry: a working concentration of 8-25 µg/mL is recommended to detect TRAIL in paraffin-embedded human brain (occipital cortex) tissue sections.

Neutralization: Measured by its ability to neutralize TRAIL/TNFSF10-induced cytotoxicity in the L929 mouse fibroblast cell line. The Neutralization Dose (ND₅₀) is typically 2-8 ng/mL in the presence of 12 ng/mL recombinant Human TRAIL/TNFSF10, a cross-linking antibody, Mouse Polyhistidine Monoclonal Antibody, and 1 µg/mL actinomycin D.

The exact concentration of antibody required to neutralize TRAIL is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity. The ND₅₀ for this antibody is defined as the concentration required to yield one-half maximal inhibition of the TRAIL-mediated activity on a responsive cell line, when TRAIL is present at a concentration high enough to elicit a maximum response.

Note: In order to obtain best results in different techniques and preparations, determination of optimal working dilutions by titration test is recommended.

References

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