3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Anti-Human Transforming Growth Factor-a
produced in goat, affinity isolated antibody

Product Number **T0563**

Product Description

Anti-Transforming Growth Factor-α (TGF-α) is developed in goat using recombinant human TGF-α (rhTGF-α) expressed in *E. coli* as immunogen. The antibody is purified using human TGF-α affinity chromatography.

Anti-Transforming Growth Factor-α may also be used in various applications including immunoblotting, immunohistochemistry, capture ELISA, and neutralization. The antibody will neutralize the biological activity of recombinant human TGF-α and can be used as a capture antibody in sandwich ELISAs. In the ELISA capture assay, less than 0.05% cross-reactivity is seen with recombinant human TGF-β1, rhAR, rhBTC, rhEGF, rhHRG-α, and rhSMDF.

Transforming Growth Factor-α, originally discovered in 1978 in conditioned medium of retrovirus-transformed fibroblasts,[1] is a protein that reversibly confers a transformed phenotype upon normal non-neoplastic cells, such as normal rat kidney (NRK) fibroblasts.[2] The transforming activity of TGF-α was later shown to require the presence of transforming growth factor-β, which potentiates the action of TGF-α via a separate receptor.[2-4] Secreted TGF-α proteins range from 5 to 20 kDa,[5] but this recombinant product from *E. coli* is a 6.0 kDa protein.[6, 7] TGF-α is similar in structure to epidermal growth factor showing a 30-35% homology in amino acid sequence with conservation of all six cysteine residues,[6] and a very similar NMR-determined three-dimensional structure.[8] TGF-a exerts its cellular action via the EGF cell-surface receptor [9, 10] and induces many of the same actions as EGF,[5, 11, 12] but is immunologically distinct from EGF.[13]

Reagent

Supplied lyophilized from a 0.2 µm filtered solution of phosphate buffered saline and 5% trehalose.

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.

Preparation Instructions

To one vial of lyophilized powder, add 1 mL of 0.2 µm-filtered phosphate buffered saline to produce a 0.1 mg/mL stock solution. If aseptic technique is used, no further filtration should be needed for use in cell culture environments.

Procedure

For capture ELISAs, the anibody can be used as the capture antibody in a human TGF-α ELISA in combination with biotinylated, human TGF-α affinity purified polyclonal detection antibody. Using plates coated with 100 µL/well of the capture antibody at 0.4 µg/mL, in combination with 100 µL/well of the detection antibody, an ELISA for sample volumes of 100 µL can be obtained. To arrive at the optimal dose range for this ELISA, set up a two-fold dilution series of the protein standard starting with 1000 pg/mL.

For neutralization, the antibody is assayed for its ability to neutralize the bioactivity of recombinant human TGF-α in a cell proliferation assay using Balb/3T3 cells.[14] In this bioassay, recombinant human TGF-α (3 ng/mL) and the antibody at concentrations of 0.001-100 ng/mL is preincubated in a 96-well plate. The total volume of 100 µl/well containing antibody, recombinant human TGF-a, and Balb/3T3 cells are incubated and then pulsed with ^{3}H-thymidine. Cells are harvested onto glass filters and the ^{3}H-thymidine incorporation into DNA is measured. The exact concentration of antibody required to neutralize human TGF-α activity is dependent on the cytokine concentration, cell type, growth conditions, and the type of activity studied.

The ND_{50} of the antibody is defined as the concentration of antibody resulting in a one-half maximal inhibition of bioactivity of recombinant human TGF-α that is present at a concentration just high enough to elicit a maximum response.

Product Profile

ELISA capture: This antibody can be used as a capture antibody. A working concentration of the capture antibody at 0.4 µg/mL (100 µl/well) is recommended.

Neutralization: The antibody has the ability to neutralize the biological activity of human TGF-a in the presence of 3 ng/mL of human TGF-a, using the Balb/3T3 cell line.

Immunoblotting: 0.1-0.2 µg/mL antibody detects recombinant human TGF-a at 50 ng/lane and 25 ng/lane under non-reducing and reducing conditions, respectively.

Immunohistochemistry: recommended working antibody concentration is 15 µg/mL after antigen retrieval using cells or tissues and a chromogenic detection system.

Note: In order to obtain the best results in various techniques and preparations, we recommend determining optimal working dilutions by titration.

Endotoxin: < 0.1 EU (endotoxin units) per 1 µg of the antibody as determined by the LAL method.

References

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