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Product datasheet for AM39040PU-N

TGF beta 1 (TGFB1) Mouse Monoclonal Antibody [Clone ID: TB21]

Product data:

Product Type:	Primary Antibodies
Clone Name:	TB21
Applications:	ELISA, FC, IHC, Neutralize, WB
Recommended Dilution:	**Flow cytometry:** Investigating cell-cell contact with regulatory $CD4^+$ T cells coexpressing membrane-bound TGF-beta or using it as a potential marker for apoptosis in COPD patients. **Western Blotting:** when used at an antibody concentration of 5-20 ng/mL visualization of 100 ng/lane of TGF-beta is obtained. **Neutralizing assay:** clone TB21 neutralizes TGF-beta activity in vitro in an inhibition assay of CCL/64 cell growth and neutralizes the growth promoting action of TGF-beta in the NRK-49F colony-forming assay. The effect of microinjection of this antibody into one blastomere of two cell stage Xenopus embryos indicated that it was able to neutralize effectively the bioactivity of TGF-b in vivo. *Recommended Dilution:* The final concentrations of Affinity-purified anti-TGF-β1 IgG used in the well were varied at range of 1-50 µg/ml (*See Weikang Shi, Jun Wu, Luxia Xu and Hsiaochien Tsung. Cell Research(1995),5,35-45*). **Immunohistochemistry:** this antibody may be used in immunohistochemical techniques to locate TGF-beta1 within tissues. Clone TB21 has been utilized in formalin-fixed paraffin-embedded sections, frozen sections, i.e., ovine ovarian tissue, breast carcinoma sections (1:1000 diluted). As a consequence of the intense staining of the erythrocytes it is possible to locate a single cell within the ovarian stroma making it useful in locating very fine capillary networks within tissue. **ELISA.** Fluorochrome-labelled antibodies are effectively formulated for direct immunofluorescent staining of human cells in flow cytometric analysis using 10 µl/10e6 leucocytes for singles and 20 µl/10e6 leucocytes in case of dual and triple combinations.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal

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Specificity: The antibody detects human TGF beta 1 (and has been used in sheep, see "Applications"). Other species not tested.
In flow cytometry this antibody detects the intracellular form of TGF-beta (so-called latent, LAP-bound or inactive TGF-beta) as well as with membrane bound TGF-beta (extracellular matrix form).
Western blotting demonstrated that this antibody reacts with the dimeric (25 kDa) and monomeric (12,5 kDa) forms of TGF-beta (active form) under both non-reducing and reducing conditions respectively.
In ELISA the antibody recognizes both human platelet-derived and recombinant TGF-beta.

Formulation: 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.3
State: Aff - Purified
State: Liquid purified Ig fraction
Stabilizer: 0.2% BSA
Preservative: 0.09% Sodium Azide
Label: Cat. No. Label EX-max (nm) / EM-max (nm):
AM39040RP-N 488, 532 / 578
AM39040PU-N Pure . /

Concentration: lot specific

Purification: Affinity chromatography

Conjugation: Unconjugated

Storage: Store the antibody undiluted at 2-8°C.
Fluorochrome labelled product is photosensitive and should be protected from light.

Stability: Shelf life: one year from despatch.

Gene Name: transforming growth factor beta 1

Database Link: Entrez Gene 7040 Human
P01137

Background: Transforming Growth Factor-Beta (TGF-beta, TGFB) was originally identified for its ability to induce phenotypic transformation of fibroblasts in diverse cell types. This protein controls proliferation, differentiation, and many other functions of various cell types.
TGFB is present on most cell types including T cells and monocytes.

Synonyms: TGFB, Transforming growth factor beta-1, TGF-beta-1

Note: 1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion.
3. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Protocol: **-A- Intracellular TGF-beta staining procedure**

1. Isolation of PBMCs

a. Collect 5-10 mL blood in a heparin or EDTA treated tube and separate PBMC using ficol-paque
b. Wash the cells once with 10 mL HBSS and centrifuge at 400 g for 15 minutes
c. Wash the cells once with 10 mL RPMI 1640 based cell culture medium and centrifuge at 300g for 10 minutes
d. Resuspend the cells in 5 mL RPMI 1640 based cell culture medium, count and adjust the concentration to 2x10e6 cells/mL.

See scheme below to calculate the amount of samples needed for stimulated cells and non-stimulated controls

2. Stimulation of PBMCs

a. Collect 5-10 mL blood in a heparin or EDTA treated tube and separate PBMC using ficol-paque
b. Add 1mL of cell suspension per well of a 24 well culture plate and add 20 µL of PMA and 10 µl Ionomycin and Monensin each
c. Incubate at 37 °C and 5% CO2 for 24 hours
d. Wash cells per well with 10 mL HBSS and centrifuge at 300 g for 10 minutes

3. Fixation

a. Discard supernatant and add 500 µL of cold (4 °C) fixation buffer
b. Incubate at room temperature for 10 minutes
c. Wash the cells with 10 mL HBSS and centrifuge at 300 g for 10 minutes

4. Permeabilization

a. Discard supernatant and add 1.5 mL of permeabilization buffer
b. Centrifuge at 200 g for 5 minutes
c. Discard supernatant and resuspend the cells in 100 µL of permeabilization buffer

5. Antibody Staining

a. Transfer the cell suspension (100 µL) to a labeled flow cytometry tube
b. Add 10 µL anti-TGF-beta-PE or isotype control IgG1-PE to the tubes and mix well
c. Incubate in the dark at 4 °C for 20 minutes
d. Add 1.5 mL of permeabilization buffer, and centrifuge at 200 g for 5 minutes
e. Discard supernatant and resuspend the cells in a sufficient amount of permeabilization buffer for flow cytometry analysis

-B- Reagents and solutions

1. Stock solutions of PMA and Ionomycin

a. Prepare separate stock solutions of 1 mg/mL PMA and 1.25 mg/mL Ionomycin in DMSO
b. Store in 10-20 µL aliquots at -20 °C
c. Prior to stimulation - dilute stock solution of PMA 1:1000 and stock solution of Ionomycin 1:50 in RPMI 1640

2. Stock solution of Monensin

a. Prepare a stock solution of 17.6 mg/mL in 98% ethanol and store in 10-20 µL aliquots at -

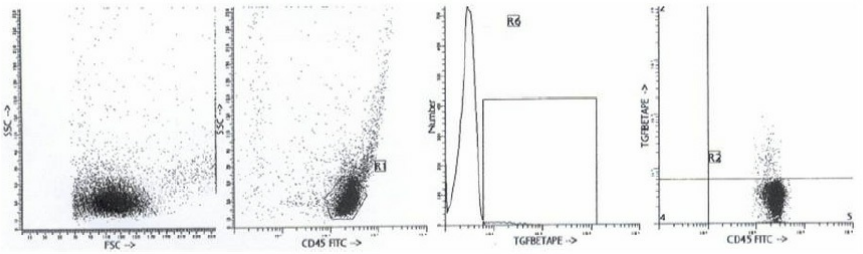
20°C
b. Prior to stimulation dilute the stock solution 1:100 in RPMI 1640
3. Permeabilization buffer
a. Dissolve 0.6 g Hepes in 150 mL demineralized water
b. Add 25 mL 10 x PBS, 2.5 mL Fetal Clone 1 Serum, 0.25 g Saponine and 0.25 g NaN3
c. Add demineralized water to a final volume of 250 mL
4. Fixation buffer
a. Dissolve 2.0 g paraformaldehyde in 5 mL demineralized water, incubate 3 hours at 70°C
b. Add a few drops of 6 M NaOH until the solution is clear
c. Dissolve 825 mg NaH2PO4 in 30 mL demineralized water, add 188 mg NaOH and 0.5 mL Fetal Clone 1 Serum
d. Add formaldehyde solution and adjust pH to 7.4 - 7.6
e. Add demineralized water to a final volume of 50 mL
f. Filtrate the solution through a 0.2 µm filter

Reagent - Amount needed for 50 tests
PMA - 1 ml
Ionomycin - 500 µl
Monensin - 500 µl
Fixation buffer - 50 ml
Permeabilization - 250 ml

Protein Families: Druggable Genome, ES Cell Differentiation/IPS, Secreted Protein, Transcription Factors

Protein Pathways: Cell cycle, Chronic myeloid leukemia, Colorectal cancer, Cytokine-cytokine receptor interaction, Dilated cardiomyopathy, Hypertrophic cardiomyopathy (HCM), MAPK signaling pathway, Pancreatic cancer, Pathways in cancer, Renal cell carcinoma, TGF-beta signaling pathway

Product images:



Staining with clone TB21 (anti-TGF-beta) is illustrated by flow cytometry analysis of stimulated mononuclear cells. Direct staining was performed using 10 µl of the PE-conjugated antibody and 100 µl stimulated cells.