PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

Anti-TGF-ß

PURE	RUO	REF	IQP-169P	50 tests
R-PE	RUO	REF	IQP-169R	50 tests

RUO ***For Research Use Only***

Description

Clone TB21

Isotype murine IgG1κ

Specificity TB21 reacts with human Transforming Growth Factor-Beta 1 (TGF-β1). In flow cytometry, this antibody detects the intracellular form of TGF-ß (so-called latent, LAP-bound or inactive TGF-ß) as well as with membrane bound TGF-ß (extracellular matrix form). Western blotting demonstrated that this antibody reacts with the dimeric (25 kDa) and monomeric (12,5 kDa) forms of TGF-ß (active form) under both non-reducing and reducing conditions respectively. This antibody recognizes both human platelet-derived and recombinant TGF-ß in ELISA.

Antigen distribution

TGF-ß is present on most cell types including T cells and monocytes.

Summary TGF-ß 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.

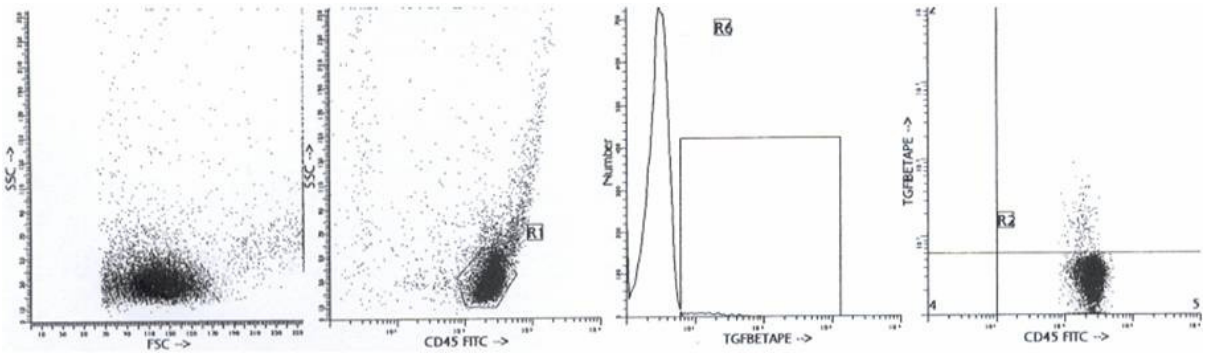
Applications Research areas - Growth Factors, Hormones and their Receptors, Angiogenesis

Flow cytometry - Investigating cell-cell contact with regulatory CD4+ T cells coexpressing membrane-bound TGF-ß 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-ß is obtained.
Neutralizing assay - TB21 antibody neutralizes TGF-ß activity in vitro, in an inhibition assay of CCL/64 cell growth and neutralizes the growth promoting action of TGF-ß 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-ß in vivo.
IHC - the monoclonal may be used in immunohistochemical techniques to locate TGF-ß 1 within tissues. 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.

Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative Data

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



Limitations

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. 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. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
3. Reagents can be used in different combinations, therefore laboratories need to become familiar performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples.
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Reagents and materials required but not supplied

1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. IQ Lyse - erythrocyte lysing solution[1] (IQP-199)
7. IQ Starfiqs – fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

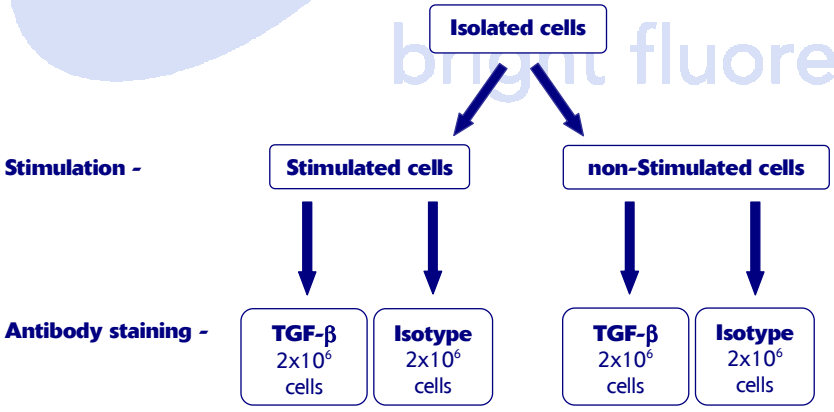
[1]M An der Grub Bio Research GmbH
Kaumberg, Austria

A - Intracellular TGF-β staining procedure

1. Isolation of PBMCs

a. Collect 5-10 ml blood in a heparin or EDTA treated tube and separate PBMC using Ficoll-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 $2x10^6$ 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 Ficoll-Paque.
b. Add 1 ml 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% CO_2 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-ß R-PE (IQP-169R) or isotype control IgG1 R-PE (IQP-191R) 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.

6. Analysis by flow cytometry

a. Analyze the cells by flow cytometry and use appropriate data processing.

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 NaN_3.
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 NaH_2PO_4 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

*) "tests" refers to one sample and its controls

Handling and Storage

Antibodies are supplied as 50 tests per vial (0.5 ml). They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN_3). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.

References

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Explanation of used symbols

	Consult instructions for use
REF	Catalogue number
	Sufficient for
IVD	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
RUO	For Research Use Only
LOT	Batch code
	Use by yyyy-mm-dd
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	Conformité Européenne (European Conformity)

		Label - tandem	Ex -max (nm)	Em -max (nm)
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
C	CyQ	PE-Cy5.18	488, 532	667
A	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695

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