Intracellular Staining Reagents

Anti-human M-CSF-Allophycocyanin Monoclonal Antibody

Catalog Number: IC2161A
Lot Number: LIM03
100 Tests

Reagent Information

Allophycocyanin (APC)-conjugated anti-human M-CSF: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone: 26786

Ig class: mouse IgG_{2A}

Storage: Reagents are stable for **twelve months** from date of receipt when stored in the dark at 2° - 8° C.

Additional Reagents Required

Paraformaldehyde Fixative - Dissolve 4.0 g of paraformaldehyde in 100 mL of sterile PBS (10 mM phosphate buffered saline, pH 7.4) by heating the solution at 56° C for about 1 hour. All solids must be fully dissolved prior to use. Store buffer at 2° - 8° C, protected from light, for no longer than 2 weeks.

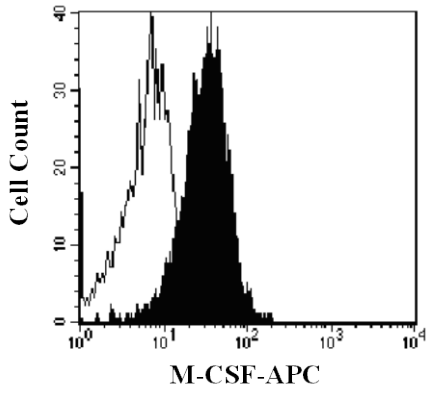
SAP buffer - Prepare a sterile solution containing 0.1% (w/v) saponin, 0.05% (w/v) NaN_3 in Hanks' Balanced Salt Solution (HBSS). Store at room temperature for no longer than 1 month.

Intended Use

The reagent is designed for flow cytometric applications intended to identify and quantitate cells expressing cytoplasmic forms of the protein recognized by the monoclonal.

Principle of the Test

Fixed cells are permeabilized, allowing conjugated antibodies access to proteins within the cell. Cells are initially subjected to a fixation step in order to minimize leakage of proteins out of the cell. The conjugated antibody is allowed to penetrate and bind to its target within the cell. Following a final wash, cells are analyzed on a flow cytometer. Flow cytometric analysis of APC conjugates will generate a signal which can be detected using 620 - 650 nm wavelength laser excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 660 - 670 nm.



Intracellular staining of Macrophages+TNFa+LPS with APC-conjugated anti-human M-CSF (Catalog # IC2161A, filled histogram) or with isotype control antibody (Catalog # IC003A, open histogram).

Sample Preparation

Intracellular staining antibodies are designed for multiparameter flow cytometric analysis of cells. To stain for surface proteins (*e.g.* CD3, CD4, CD8) in addition to the intracellular protein, we recommend that the investigator determine whether the fixation and permeabilization steps adversely affect the surface protein. If so, surface staining of cells prior to fixation and permeabilization is recommended.

For intracellular staining, cells must first be fixed and permeabilized. Use of 4% paraformaldehyde in PBS as a fixative is recommended. Other formulations or tissue fixatives may affect the staining properties of the monoclonal antibody. For permeabilization, 0.1% saponin in a balanced salt solution is effective in facilitating antibody entry into cells. Due to the reversible nature of cell membrane permeabilization, saponin **must** be included in all buffers used (i.e. both the staining and washing steps).

Sample Staining

1. Harvest cells, and wash twice in cold HBSS or PBS by spinning at 200 x g for 7 minutes.
2. If cells are to be surface stained, follow the staining procedure indicated by the antibody manufacturer.
3. Up to 1 x 10^6 cells were resuspended in 0.5 mL of cold Flow Cytometry Fixation Buffer (Catalog # FC004) and incubated at room temperature for 10 minutes.
4. Vortex the cells intermittently in order to maintain a single cell suspension. Following fixation, wash the cells twice in HBSS or PBS by centrifuging at 200 x g for 7 minutes.
5. Harvest the cell pellet from each tube and resuspend in 2 mL of SAP buffer.
6. Centrifuge the cells at 200 x g for 7 minutes.
7. Decant the supernatant, ensuring that approximately 200 μL of SAP buffer remains in the tube.
8. Gently resuspend the cells in the remaining SAP buffer and add 10 μL (or a previously titrated amount) of antibody conjugate.
9. Briefly vortex the tube and incubate for 30 - 45 minutes at room temperature **in the dark**.
10. Wash the cells twice using 2 mL of SAP buffer each time. Centrifuge as in step 6.
11. Resuspend the cells in each tube with 200 - 400 μL of PBS for final flow cytometric analysis.

FOR RESEARCH USE ONLY. NOT FOR USE IN HUMANS.

R&D Systems, Inc.
1-800-343-7475

Background Information

Human macrophage-colony stimulating factor (hM-CSF or CSF-1) is a pleiotropic cytokine and hematopoietic growth factor (1 - 3). Alternative splicing of the mRNA and some post-translational modifications yields three different isoforms of the protein: a) a 44 kDa, cell surface-associated, homodimeric glycoprotein, b) a homo- or heterodimeric proteoglycan, up to 150 kDa in size, anchored in the extracellular matrix, and c) an 85 kDa homodimeric, secreted, soluble form (2, 3). M-CSF is produced by a variety of normal cell types such as bone marrow-derived stromal cells (4), endothelial cells (5), fibroblasts (6), monocytes (7), and activated T and B cells (8, 9). M-CSF is also produced by several tumor cell types including myeloid leukemias (10), renal cell carcinoma (11) and adenocarcinomas of the lung, breast, ovary and pancreas (12 - 14).

M-CSF activity is mediated by a cell surface tyrosine kinase receptor that is the product of the *c-fms* proto-oncogene (15, 16). The M-CSF receptor is found primarily on monocytes (17), however, it is also expressed on pre-B cells (18), osteoclasts (19), placental and uterine tissues (20), neurons and microglial cells (21). Aberrant expression of M-CSF receptors and over-production of M-CSF protein are hallmarks in a number of neoplasms including AML, breast and ovarian cancer (22, 13, 14). M-CSF was first identified as a growth factor required for the differentiation, survival, and proliferation of monocytes/macrophages (1). M-CSF also plays a role in inflammation, bone morphology, fertility and pregnancy (2, 22). Further studies involving M-CSF may yield important information relating to a number of biologic processes.

References

1. Stanley, E.R. (1983) J. Cell. Biochem. **21**:151.
2. Fixe, P. and V. Praloran (1998) Cytokine **10**:32.
3. Flanagan, A.M. and C.S. Lader (1998) Curr. Opin. Hematol. **5**:181.
4. Fibbe, W.E. *et al.* (1988) Blood **71**:430.
5. Seelentag, W.K. *et al.* (1987) EMBO J. **6**:2261.
6. Tushinski, R.J. *et al*. (1982) Cell **28**:71.
7. Horiguchi, J. *et al*. (1988) Mol. Cell. Biol. **8**:3951.
8. Praloran, V. *et al*. (1990) Leukemia **4**:411.
9. Reisbach, G. *et al*. (1989) Blood **74**:959.
10. Tang, S.S. *et al*. (2001) Leuk. Res. **25**:1107.
11. Gerharz, C.D. *et al*. (2001) Urology **58**:821.
12. Roth, P. and E.R. Stanley (1992) Curr. Top. Microbiol. Immunol. **181**:141.
13. Yee, L.D. and L. Liu (2000) Anticancer Res. **20**:4379.
14. Toy, E.P. *et al*. (2001) Gynecol. Oncol. **80**:194.
15. Sherr, C.J. *et al*. (1985) Cell **41**:665.
16. Coussens, L. *et al*. (1986) Nature **32**:277.
17. Arceci, R.J. *et al*. (1989) Preoc. Natl. Acad. Sci. USA **86**:8818.
18. Lu, L. and D.G. Osmond (2001) Exp. Hematol. **29**:596.
19. Felix, R. *et al*. (1994) J. Cell. Biochem. **55**:340.
20. Pampfer, S. *et al*. (1992) Biol. Reprod. **46**:48.
21. Brosnan, C.F. *et al*. (1993) Adv. Neurol. **59**:329.
22. Motoyoshi, K. (1998) Int. J. Hematol. **67**:109.

Technical Notes

Appropriate negative controls should be run to verify specificity and rule out background staining. An irrelevant antibody of the same isotype and concentration should be run to aid in setting quadrant statistics. Antibody binding can also be blocked with a pre-incubation of excess target protein (10X molar excess should give > 90% inhibition of staining). In some cases, however, enhanced staining has been observed. This may be due to detection of protein binding cell surface receptors. Unlabeled antibody may also be used as a control. An excess of unlabeled antibody in the presence of labeled antibody should completely inhibit staining.

Production of certain proteins varies between different populations of cells as well as among cells within the same population. Some cells may produce a protein at concentrations below the detection limits of the monoclonal antibody. The investigator is encouraged to determine optimal titers for each antibody conjugate. The use of either monensin or brefeldin A, which act to block intracellular protein transport and result in an accumulation of protein in the Golgi, will enhance detection of the target protein in low level secreting cells.

Some antibodies are sensitive to the conformational structure of their target epitope. Fixation of cells using extremely harsh conditions or for a prolonged period of time may alter the target epitope and render it unrecognizable to the antibody. Strict attention to the recommended fixation conditions must be followed in order to generate consistent results.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.