



Catalog Number:	FC15016	Species Reactivity:	Human
Product Type	Mouse Monoclonal Protein G purified IgG ₁ Antibody. Clone #: 132507	Format:	Carboxyfluorescein (CFS)-conjugated anti-human Ret: Supplied as 25 µg of antibody in 1 mL PBS containing 0.1% sodium azide.
Size:	100 Tests	Note:	This reagent 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.
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.		
Storage:	2 -8° C		

Application Notes and Protocol

Background Information

Ret is a receptor tyrosine kinase that associates with several different ligand-binding subunits of the GDNF (glial cell derived neurotrophic factor) family acting as the signaling component in the receptor complex for ligands including GDNF, neurturin, artemin and persephin.

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) NaN₃ in Hanks' Balanced Salt Solution (HBSS). Store at room temperature for no longer than 1 month.

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 fluorescein conjugates will generate a signal, which can be detected using 488 nm wavelength laser excitation and monitoring emitted fluorescence with a detector, optimized to collect peak emissions at 515 - 545 nm.

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).

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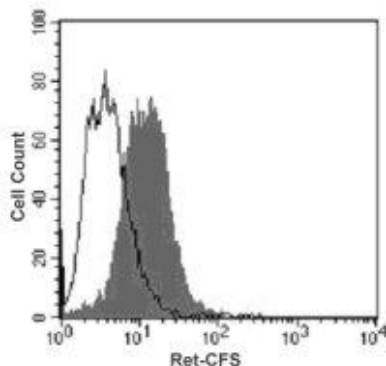


Figure: Intracellular staining of SH-SY5Y cells with CFS-conjugated anti-human Ret (filled histogram) or with isotype control antibody (open histogram).

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. Resuspend a maximum of 5×10^5 washed (or surface stained) cells in 0.5 mL of cold 4% paraformaldehyde fixative and incubate 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 μ L of SAP buffer remains in the tube.
8. Gently resuspend the cells in the remaining SAP buffer and add 10 μ L (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 μ L of PBS for final flow cytometric analysis.

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.

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