ADVANCED TARGETING SYSTEMS

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Antibody to Serotonin Transporter (SERT)

MOUSE MONOCLONAL

Catalog Number: AB-N40
Quantity: 100 micrograms
Format: PBS (0.14 M Sodium Chloride; 0.003 M Potassium Chloride; 0.002 M Potassium Phosphate; 0.01 M Sodium Phosphate; pH 7.4), no preservative.
Host: Mouse
Isotype: IgG2b
Clone: 15A2
Immunogen: peptide from the fourth extracellular domain of the rat SERT

Background:
The serotonin (5HT) transporter (5HTT, SERT) is the major determinant of serotonin inactivation following release at synapses, is the site of action for many tricyclic antidepressants and the SSRIs (serotonin-selective reuptake inhibitor), and is also targeted by a number of psychostimulants including cocaine, methylphenidate, and MDMA 'ecstasy.' SERT is produced from a single gene and is expressed in both the CNS and GI system. The serotonergic system is known to modulate mood, emotion, sleep and appetite and thus is implicated in the control of numerous behavioral and physiological functions. Decreased serotonergic neurotransmission has been proposed to play a key role in the etiology of depression. Recent findings suggest that SERT might be linked to both neurotic and sexual behavior as well as to obsessive-compulsive disorder (OCD). The concentration of synaptic serotonin is controlled directly by its reuptake into the pre-synaptic terminal and, thus, drugs blocking serotonin transport have been successfully used for the treatment of depression. SERT first binds a sodium ion, followed by serotonin, and then a chloride ion. The transporter then flips inside the cell, releasing serotonin. A potassium ion binds, and the transporter flips back out, ready to receive another serotonin molecule.

Specificity and Preparation:
This antibody recognizes cells that express SERT in rat, human, and mouse. The immunogen is a peptide from the fourth extracellular domain of the rat SERT. This antibody was produced in tissue culture supernatants and purified by 50% $(NH_4)SO_4$ cut followed by protein A column chromatography.

Usage and Storage:
Applications include immunocytochemistry (5 μg/ml; ATS in-house), flow cytometry (Fig 1: 5 μg per 2 x 10^6 cells, ATS in-house; Fig 2: 1-5 μg/ml), targeting (targeting agent in Anti-SERT-SAP, Cat. #IT-23).
Store the antibody at -20°C in undiluted aliquots. Avoid repeated freezing and thawing. Gently spin down material before use; 5-10 seconds in a microfuge should be adequate.

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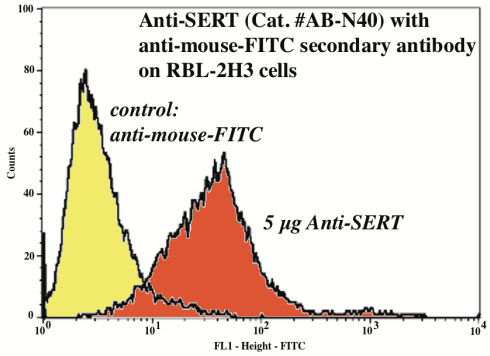


FIGURE 1: The rat basophilic leukemic cell line RBL-2H3 (2 x 10^6 cells per sample) was incubated with 5 μg of anti-SERT clone 15A2 for 1 hour. The anti-mouse-FITC secondary antibody was applied at 2 μg per sample for 30 minutes. The control in this figure is secondary antibody alone. Data analysis was done with CellQuest software.

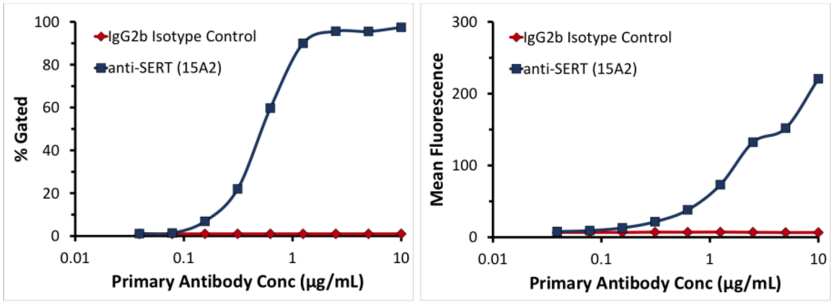
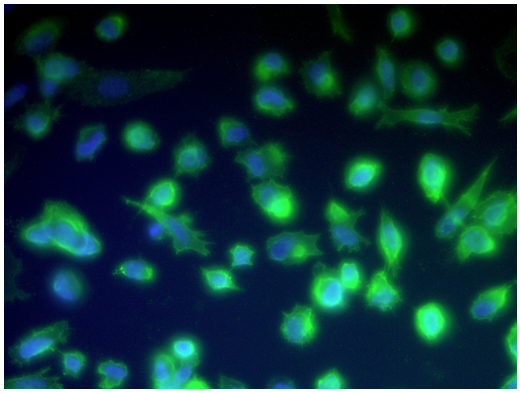


FIGURE 2: Sodium citrate anticoagulated human platelet-rich plasma (PRP) was fixed with 1% formaldehyde (30 min) and permeabilized with 0.2% Triton X-100. PRP was then incubated with a nonspecific IgG2b antibody (isotype control) or the anti-SERT monoclonal antibody (15A2) for 30 min at room temperature, followed by a 30 min incubation with 20 μg/ml goat anti-mouse PE-conjugated secondary antibody. Samples were analyzed on a BD FACSCalibur and platelets were identified by their characteristic light scatter properties. The % Gated (left) represents the percentage of platelets staining positive for 15A2 beyond the isotype control. The Mean Fluorescence (right) represents the mean fluorescence of individual platelets.
Courtesy of Michelle A. Berny-Lang, Ph.D. from the Center for Platelet Research Studies, Boston Children's Hospital and Harvard Medical School, Boston, MA.



Immunofluorescent staining of RBL-2H3 cells, a rat basophilic leukemia cell line, with a mouse monoclonal antibody directed against the serotonin transporter (SERT). Cells were fixed with paraformaldehyde and blocked prior to staining with primary at 10 µg/ml followed by goat anti-mouse-FITC secondary at 50 µg/ml and DAPI at 5 µg/ml for nuclear staining.
Images were obtained using a 40x objective and a Leica DM IL fluorescent microscope. SERT staining is represented in green and nuclear staining is represented in blue.