Human ABCG2 Biotinylated Antibody

Monoclonal Mouse IgG_{2B} Clone # 5D3

Catalog Number: BAM995

DESCRIPTION

Species Reactivity	Human
Specificity	Detects human ABCG2.
Source	Monoclonal Mouse IgG_{2B} Clone # 5D3
Purification	Protein A or G purified from ascites
Immunogen	3T3 cells transduced with human ABCG2
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with BSA as a carrier protein. See Certificate of Analysis for details.

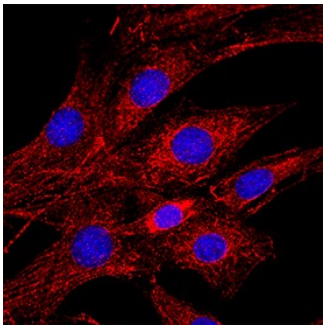
APPLICATIONS

Please Note: *Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.*

	Recommended Concentration	Sample
Flow Cytometry	2.5 µg/10^6 cells	Human MCF-7 cell line
Immunocytochemistry	5-25 µg/mL	Immersion fixed A549 human lung carcinoma cell line

DATA

Immunocytochemistry



ABCG2 in A549 Human Cell Line. ABCG2 was detected in immersion fixed A549 human lung carcinoma cell line using Mouse Anti-Human ABCG2 Biotinylated Monoclonal Antibody (Catalog # BAM995) at 5 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Streptavidin (red; Catalog # NL999) and counterstained with DAPI (blue). Specific staining was localized to cytoplasm. Staining was performed using our protocol for Fluorescent ICC Staining of Non-adherent Cells.

PREPARATION AND STORAGE

Reconstitution	Reconstitute at 0.5 mg/mL in sterile PBS.
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 12 months from date of receipt, -20 to -70 °C as supplied. • 1 month, 2 to 8 °C under sterile conditions after reconstitution. • 6 months, -20 to -70 °C under sterile conditions after reconstitution.

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BACKGROUND

Hematopoietic stem cells are known to express a membrane transporter molecule, known as P-glycoprotein (Pgp), that is encoded by the multidrug resistance gene 1 (MDR1) (1, 2). Expression of Pgp appears to confer a proliferative advantage to stem cells through its anti-apoptotic effects (3, 4). An additional transporter molecule known as ABCG2 (ATP-binding cassette gene 2) or Bcrp1 (Breast cancer resistance protein 1), first identified in a breast cancer cell line (5), is expressed on stem cells (6). ABCG2 belongs to a family of molecules that span the cell membrane six times and can exist as either homo or hetero dimers linked by a short intracellular flexible linker region that plays an important role in the efflux of a wide range of substrates (7, 8). Although these transporter molecules have initially been thought to play a role in drug resistance, they have been found to have utility in better characterizing primitive stem cells. For example, the “side-population” of hematopoietic stem cells, characterized by their inability to retain high levels of the intracellular staining dyes Hoechst 33342 and Rhodamine 123, has been found to express high levels of ABCG2. Of interest is the observation that ABCG2 function has been linked to the efflux of the Hoechst dye (6). Furthermore, there is now evidence that this monoclonal can be used as a cell surface marker to identify hematopoietic stem cells within the bone marrow fraction of lineage negative cells (6). The expression of ABCG2 appears greatest on $CD34^-$ cells and is downregulated with the acquisition of CD34 on the cell surface (6).

References:

1. Chaudhary, P.M. and I.B. Roninson (1991) Cell **66**:85.
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4. Johnstone, R.W. *et al.* (1999) Blood **93**:1075.
5. Doyle, L.A. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:15665.
6. Zhou, S. *et al.* (2001) Nat. Medicine **7**:1028.
7. Hrycyna, C.A. *et al.* (1998) Biochem. **37**:13660.
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