

Monoclonal Anti-human ABCG2-Alexa Fluor® 700

Catalog Number: FAB995N

Lot Number: ADOW01

100 Tests

Reagents Provided

Alexa Fluor® 700-conjugated mouse monoclonal anti-human ABCG2: Supplied as 10 µg of antibody in 0.5 mL saline containing up to 0.5% BSA and 0.09% sodium azide.

Clone #: 5D3

Isotype: mouse IgG_{2B}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

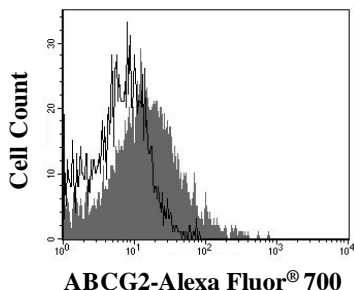
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing ABCG2 within a population and qualitatively determine the density of ABCG2 on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with 3T3 cells transduced with human ABCG2. The IgG fraction of the tissue culture supernatant was purified by Protein A or G affinity chromatography. The purified antibody was then conjugated to Alexa Fluor® 700 fluorochrome. Cell surface expression of ABCG2 is determined by flow cytometry using 675-700 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at around 723 nm.



MCF-7 cells were stained with Alexa Fluor® 700-conjugated anti-human ABCG2 (Catalog # FAB995N; filled histogram) or Alexa Fluor® 700-conjugated isotype control (Catalog # IC0041N; open histogram).

Background Information

Hematopoietic stem cells are known to express a membrane transporter molecule, known as P-glycoprotein (Pgp), that is encoded by the multi-drug 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 homodimers or heterodimers linked by a short intracellular flexible linker region that plays an important role in the efflux of a wide range of substrates.^{7,8}

References

1. Chaudhary, P.M. & I.B. Roninson (1991) *Cell* **66**:85.
2. Sorrentino, B.P. *et al.* (1995) *Blood* **86**:491.
3. Pallis, M. & N. Russell (2000) *Blood* **95**:2897.
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. Med.* **7**:1028.
7. Hrycyna, C.A. *et al.* (1998) *Biochem.* **37**:13660.
8. Bunting, K.D. (2002) *Stem Cells* **20**:11.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using MCF-7 cells.

1. Cells may be Fc-blocked with 1 µg of human IgG/10⁵ cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 5 µL of conjugated antibody was added to up to 1 x 10⁶ cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with Alexa Fluor® 700-labeled mouse IgG_{2B} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

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.

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