Miltenyi Biotec

# Anti-ABCG2 (CD338) MicroBeads
## human

Order no. 130-107-680

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## Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

## 1. Description

**This product is for research use only.**

| | |
|---|---|
| **Components** | 2 mL Anti-ABCG2 (CD338) MicroBeads, human: MicroBeads conjugated to monoclonal anti-human ABCG2 (CD338) antibodies (isotype: mouse IgG2bκ). |
| **Capacity** | For $10^9$ total cells, up to 100 separations. |
| **Product format** | Anti-ABCG2 (CD338) MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| **Storage** | Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. |

### 1.1 Principle of the MACS® Separation

First, the ABCG2 (CD338)$^+$ cells are magnetically labeled with Anti-ABCG2 (CD338) MicroBeads. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled ABCG2 (CD338)$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of ABCG2 (CD338)$^+$ cells. After removing the column from the magnetic field, the magnetically retained ABCG2 (CD338)$^+$ cells can be eluted as the positively selected cell fraction.

### 1.2 Background information

ABCG2, also known as CD338 or BCRP1, is a multi-drug resistance (MDR) family protein that is a member of ATP-binding cassette (ABC) transporters. ABC proteins transport various molecules across extracellular and intracellular membranes. It functions as a xenobiotic transporter which may play a role in multi-drug resistance to chemotherapeutic agents[1]. ABCG2 is highly expressed on primitive side population (SP) stem cells. This SP phenotype is based on the efflux of fluorescent dyes such as Rhodamine 123 and Hoechst 33342 by ABCG2. It was shown that expression of the ABCG2 gene is a conserved feature of stem cells from a wide variety of sources[2].

### 1.3 Applications

- Positive selection or depletion of cells expressing human ABCG2 (CD338) antigen.

### 1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, and 0.5% bovine serum albumin (BSA). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- MACS Columns and MACS Separators: ABCG2 (CD338)$^+$ cells can be enriched by using LS or XS or depleted with the use of LD, CS, or D Columns. As most cells show a low expression level of ABCG2, the use of an LS Column is recommended for optimal recovery during enrichment. Positive selection or depletion can also be performed by using the autoMACS® Pro Separator.

| Column | Max. number of labeled cells | Max. number of total cells | Separator |
|---|---|---|---|
| **Positive selection** | | | |
| LS | $2\times10^7$ | $4\times10^7$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS II |
| XS | $10^9$ | $2\times10^{10}$ | SuperMACS II |
| **Depletion** | | | |
| LD | $1.5\times10^7$ | $3\times10^7$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS II |
| CS | $2\times10^8$ | | VarioMACS, SuperMACS II |
| D | $10^9$ | | SuperMACS II |
| **Positive selection or depletion** | | | |
| autoMACS | $5\times10^7$ | $10^8$ | autoMACS Pro |

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ II Separators. For details refer to the respective MACS Separator data sheet.


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- Labeling Check Reagent conjugated to, e.g., PE (# 130-095-228). Due to the low expression level of ABCG2 on most cells it is not possible to use direct ABCG2 fluorochrome-conjugates for fluorescently staining of already ABCG2 (CD338) MicroBead–labeled cells. For evaluation of MACS Separations by flow cytometry or fluorescence microscopy, use the Labeling Check Reagent.
- FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.

## 2. Protocol

### 2.1 Sample preparation

When working with solid tissue, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociators.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

▲ Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation or the Dead Cell Removal Kit (# 130-090-101).



### 2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to $10^7$ total cells. When working with fewer than $10^7$ cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for $2\times10^7$ total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Add 70 µL of buffer per $10^7$ total cells to the cell pellet.
4. Add 10 µL of FcR Blocking Reagent, human per $10^7$ total cells.
5. Add 20 µL of Anti-ABCG2 (CD338) MicroBeads per $10^7$ total cells.
6. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend up to $10^8$ cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** For depletion with LD Columns, resuspend up to $1.25\times10^8$ cells in 500 µL of buffer.
9. Proceed to magnetic separation (2.3).



### 2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of ABCG2 $(CD338)^+$ cells. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

**Magnetic separation with LS Columns**

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to the respective MACS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

**Magnetic separation with XS Columns**

For instructions on the column assembly and the separation refer to the XS Column data sheet.

**Depletion with LD Columns**

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to the LD Column data sheet.
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 2×1 mL of buffer. Collect total flow-through; this is the unlabeled cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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**Depletion with CS Columns**

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator. For details refer to the CS Column data sheet.
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way stopcock of the assembled column. For details refer to the CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 30 mL buffer from the top. Collect total flow-through; this is the unlabeled cell fraction.

**Depletion with D Columns**

For instructions on the column assembly and separation refer to the D Column data sheet.

**Magnetic separation with the autoMACS® Pro Separator**

▲ Refer to the respective user manual for instructions on how to use the autoMACS® Pro Separator.

▲ Buffers used for operating the autoMACS Pro Separator should have a temperature of ≥10 °C.

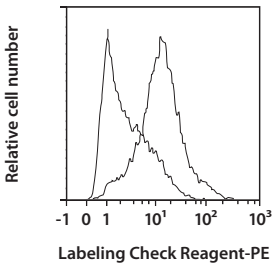
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

   **Positive selection:**
   **PosselS** for highest recovery
   or
   **PosselD** for highest purity
   Collect positive fraction in row C of the tube rack.

   **Depletion: DepleteS**
   Collect negative fraction in row B of the tube rack.

## 3. Example of a separation using Anti-ABCG2 (CD338) MicroBeads

The ABCG2$^+$ subpopulation of human breast cancer cells (MCF7) was isolated using Anti-ABCG2 (CD338) MicroBeads, an LS Column, and a QuadroMACS™ Separator. Cells were fluorescently stained with Labeling Check Reagent-PE (# 130-095-228) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.




## 4. References

1. Doyle, L. A. *et al.* (1999) A multidrug resistance transporter from human MCF-7 breast cancer cells. Proc. Natl. Acad. Sci. U. S. A. 95 (26): 15665–15670.
2. Zhou, S. *et al.* (2001) The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat. Med. 7 (9): 1028–1034.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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