Miltenyi Biotec

Anti-ErbB-2 MicroBeads
human

Order no. 130-090-482

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1. Description

Components	2 mL Anti-ErbB-2 MicroBeads, human: MicroBeads conjugated to monoclonal anti-human erbB-2 (HER-2/neu) antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells.
Product format	Anti-ErbB-2 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 erbB-2^+ cells are magnetically labeled with Anti-ErbB-2 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 erbB-2^+ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of erbB-2^+ cells. After removing the column from the magnetic field, the magnetically retained erbB-2^+ cells can be eluted as the positively selected cell fraction.

1.2 Background information

The erbB-2 antigen, also known as HER-2/neu, belongs to the family of human epithelial growth factor receptors. It is a 185 kD transmembrane glycoprotein receptor with tyrosine kinase activity.[1,2] ErbB-2 is overexpressed in 25–30% of human breast cancers[3] as well as in various other tumors ,e.g., ovarian, prostate, lung, colorectal, pancreatic, or gastric cancers. The erbB-2 protein has been detected in both, normal tissues and various cancer cell lines. [4,5]

Anti-ErbB-2 MicroBeads can be used for the enrichment of disseminated epithelial erbB-2 expressing tumor cells from peripheral blood or hematopoietic tissue. Enriched erbB-2^+ tumor cells can be phenotypically or genotypically characterized, or directly taken into culture.

1.3 Applications

- Positive selection or depletion of cells expressing human erbB-2^+ antigen.
- Isolation or depletion of erbB-2^+ cells from non-epithelial tissue (e.g. peripheral blood, leukapheresis harvests, or single cell suspensions from lymph nodes or bone marrow).
- Enrichment of disseminated carcinoma cells from peripheral blood, bone marrow, and lymphoid tissue of patients with epithelial cancer for subsequent phenotypic or genotypic analysis.
- Magnetic immobilization of erbB-2^+ cells on a MACS Column for solid phase intracellular staining procedures using the Inside Stain Kit (# 130-090-477).

1.4 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). 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: ErbB-2^+ cells can be enriched by using MS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

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

- FcR Blocking Reagent (# 130-059-901).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.

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- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details see 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 2×10^7 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, # 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. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Wash cells by adding 10–20× labeling volume of buffer and centrifuge at 300×g for 10 minutes.
2. Aspirate supernatant completely.
3. Start with 5×10^7 total cells. Resuspend cell pellet in 300 µL of buffer.
4. Add 100 µL FcR Blocking (# 130-059-901) per 5×10^7 total cells to block non-specific binding of the Anti-ErbB-2 MicroBeads.

   ▲ Note: To block FcR mediated non-specific labeling of non-epithelial cells it is strongly recommended to use FcR Blocking Reagent.
5. Add 100 µL of Anti-ErbB-2 MicroBeads per 5×10^7 total cells, the final labeling volume is 500 µL per 5×10^7total cells.
6. Mix well and incubate for 30 minutes in the refrigerator (2–8°).
7. Wash cells by adding 10–20× the labeling of buffer and centrifuge at 300×g for 10 minutes.
8. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. 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 erbB-2^+ cells. For details see table in section 1.4.

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

Magnetic separation with MS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension in 500–1000 µL of buffer onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase the purity of erbB-2^+ cells, the eluted fraction can be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with the autoMACS™ Pro Separator or the autoMACS™ Separator

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

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

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS™ Pro Separator

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.

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3. For the selection of the appropriate program for a standard separation please refer in the respective user manual.

   For a separation of samples with less than 10% of erbB-2$^+$ cells use the following program:

   Positive selection: "POSSELD"
   Collect positive fraction in row C of the tube rack.

Magnetic separation with the autoMACS™ Separator

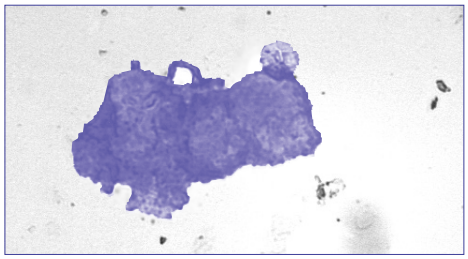
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and pos1 or pos2.
3. For the selection of the appropriate program for a standard separation please refer in the respective user manual.

   For a separation of samples with less than 10% of erbB-2$^+$ cells use the following program:

   Positive selection: "POSSELD"
   Collect positive fraction from outlet pos2.

3. Example of a separation using the Anti-ErbB-2 MicroBeads

ErbB-2$^+$ tumor cells were isolated from human PBMCs of a patient with breast cancer using the Anti-ErbB-2 MicroBeads.



PBMCs from a patient with primary breast cancer were magnetically labeled using Anti-ErbB-2 MicroBeads. A cluster of breast cancer cells was detected in the enriched fraction by immunocytochemical staining following the solid phase intracellular staining protocol using the Inside Stain Kit (# 130-090-477) in combination with Anti-Cytokeratin-Phosphatase (# 130-090-462).

4. References

1. Yamamoto, T. *et al.*(1986) Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. Nature 319: 230–234.
2. Hung, M.C. and Lau, Y.K. (1999) Basic science of HER-2/NEU: a review. Semin. Oncol. 26: 51–59.
3. Slamon, D.J. *et al.* (1989) Studies of the HER-2/neu protooncogene in human breast and ovarian cancer. Science 244: 707–712.
4. Akiyama, T. *et al.* (1986) The product of the human c-erbB-2 gene: A 185-kilodalton glyocoprotein with tyrosine kinase activity. Science 232: 1644–1646.
5. Gullick, W.J. *et al.* (1987) Expression of the c-erbB-2 protein in normal and transformed cells. Int. J. Cancer 40: 246–254.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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.

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