Miltenyi Biotec

Anti-LGR5 MicroBeads
human

Order no. 130-104-072

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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-LGR5 MicroBeads, human : MicroBeads conjugated to monoclonal anti-human LGR5 antibodies (rat IgG2b).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-LGR5 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 LGR5$^+$ cells are magnetically labeled with Anti-LGR5 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 LGR5$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of LGR5$^+$ cells. After removing the column from the magnetic field, the magnetically retained LGR5$^+$ cells can be eluted as the positively selected cell fraction.

1.2 Background information

LGR5 associates with Wnt-receptors and acts as R-spondin receptor thereby playing a central role in the modulation of Wnt/β-catenin signaling in normal and neoplastic stem cells.[1,2] Initially described as a highly specific marker for stem cells in the small intestine, colon, hair follicle, stomach, and during kidney development[3,4,5,6], LGR5-positive cells were also shown to be crucial during the development and progression of cancer. It was shown that LGR5$^+$ crypt stem cells are the cells-of-origin in intestinal cancer and that CSCs in human colorectal cancer can be identified and isolated based on LGR5 expression.[7,8]

1.3 Applications

- Positive selection or depletion of cells expressing human LGR5 antigen.

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).
  Always use freshly prepared buffer. Do **not use** autoMACS Running Buffer or MACSQuant® Running Buffer as they contain a small amount of sodium azide that could affect the results.

  ▲ **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.

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- MACS Columns and MACS Separators: LGR5$^+$ 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 LGR5, 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	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
CS	2×10^8		VarioMACS, SuperMACS II
D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	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.

- (Optional) Fluorochrome-conjugated CD44 antibodies for flow cytometric analysis, e.g., CD44-APC (# 130-095-177). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- Labeling Check Reagent conjugated to, e.g., PE. Due to the low expression level of LGR5 on most cells it is not possible to use direct LGR5 fluorochrome-conjugates for fluorescently staining of already Anti-LGR5 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) gentleMACS Dissociator (# 130-093-235), gentleMACS Octo Dissociator (# 130-095-937), or gentleMACS Octo Dissociator with Heaters (# 130-096-427) and gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (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 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 lysed blood, prepare a single-cell suspension using standard methods.

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

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.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, 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-LGR5 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. (Optional) Add staining antibodies, e.g., 10 µL of Labeling Check Reagent-PE (# 130-095-228), and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).

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9. Resuspend up to 4×10^7 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 1.25×10^8 cells in 500 µL of buffer.

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 LGR-5^+ 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 the 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.

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 the following program:

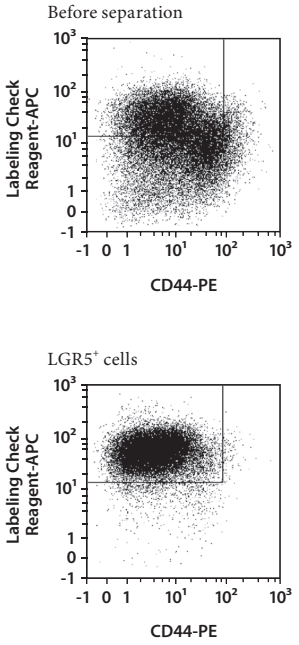
   Positive selection: Possels
   Collect positive fraction in row C ot the tube rack.

   Depletion: Depletes

   Collect negative fraction in row B of the tube rack.

3. Example of a separation using Anti-LGR5 MicroBeads

The LGR5$^+$ subpopulation of human colorectal adenocarcinoma cells (LoVo) was isolated using Anti-LGR5 MicroBeads, an LS Column, and a QuadroMACS™ Separator. Cells were fluorescently stained with Labeling Check Reagent-PE (# 130-095-228) and CD44-APC (# 130-095-177) 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

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8. Kemper, K. *et al.* (2012) Monoclonal antibodies against lgr5 identify human colorectal cancer stem cells. Stem Cells 30(11): 2378–2386.

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.

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