# Anti-Biotin MultiSort Kit

Order No. 130-091-256

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## 1. Description

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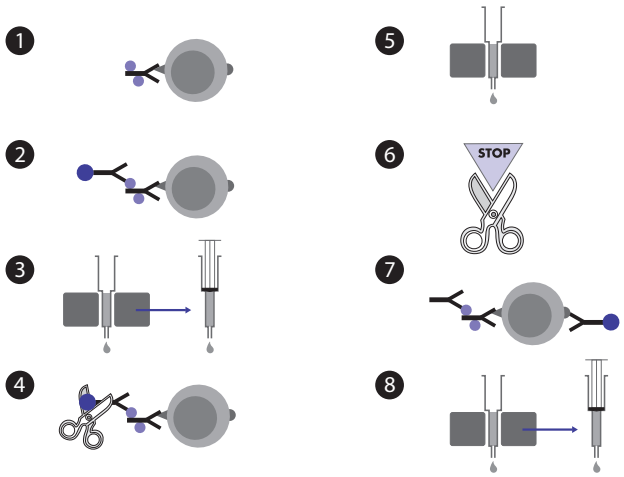
|---|---|
| **Components** | **2 mL Anti-Biotin MultiSort MicroBeads:** MultiSort MicroBeads conjugated to monoclonal mouse Anti-Biotin antibodies (isotype: mouse IgG1; clone: Bio3-18E7.2)<br>**1 mL MultiSort Release Reagent**<br>**2 mL MultiSort Stop Reagent** |
| **Size** | For 1–5x$10^9$ total cells |
| **Product format** | The Anti-Biotin MultiSort MicroBeads are supplied as a suspension containing 0.1% gelatine and 0.05% sodium azide.<br>The MultiSort Release Reagent is supplied in a solution containing 0.1% gelatine and 0.05% sodium azide.<br>The MultiSort Stop Reagent is supplied in a solution containing 0.05% sodium azide. |
| **Storage** | Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label. |

### 1.1 Principle of Anti-Biotin MultiSort Kit




1. Cells are labeled with biotinylated primary antibodies.
2. Cells are magnetically labeled with Anti-Biotin MultiSort MicroBeads.
3. Labeled cells are enriched by using MACS® Columns and Separators.
4. The MultiSort Release Reagent releases the MultiSort MicroBeads from the cells.
5. Remaining magnetically labeled cells are removed by using MACS Columns and Separators. The released cells are collected in the flow through.
6. The release reaction is stopped by adding MultiSort Stop Reagent to the flow through fraction.
7. Magnetic labeling of a second cell surface marker is performed by using MACS MicroBeads.
8. Labeled cells are enriched by using MACS Columns and Separators.

### 1.2 Background and product applications

The Anti-Biotin MultiSort Kit is an indirect magnetic labeling system which allows the sorting of cells according to multiple surface markers. Cells are first labeled with a biotinylated primary antibody followed by magnetic labeling with Anti-Biotin MultiSort MicroBeads. Following enrichment of the biotin-labeled cells, using a MACS Column and Separator, the magnetic particles are removed from the cells by using MultiSort Release Reagent. This allows for a second magnetic labeling and separation of the cells for another surface marker of interest. Magnetic labeling for the second marker is achieved by using either direct or indirect magnetic labeling with MACS® MicroBeads. For indirect labeling, Anti-FITC, Anti-PE or Anti-APC MicroBeads are recommended.



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### 1.3 Reagent and instrument requirements

- Buffer (degassed): PBS (phosphate buffered saline) pH 7.2, supplemented with 0.5% BSA (bovine serum albumin) and 2 mM EDTA. Keep buffer cold (4–8 °C).

  ▲ **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 gelatine, human serum albumin, human serum or fetal calf serum. Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- biotinylated antibody, peptide or ligand.
- (Optional) Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC ( # 130-090-856) for fluorescent staining.
- MACS Columns and MACS Separators:

**Table 1: MACS Columns and MACS Separators**

| Column | max. number of labeled cells | max. number of total cells | Separator |
|---|---|---|---|
| MS | $10^7$ | $2x10^8$ | MiniMACS, OctoMACS, VarioMACS, SuperMACS |
| LS | $10^8$ | $2x10^9$ | MidiMACS, QuadroMACS, VarioMACS, SuperMACS |
| autoMACS | $2x10^8$ | $4x10^9$ | autoMACS |

▲ **Note:** Column adapters are required to insert MS or LS Columns into VarioMACS or SuperMACS. For details, see MACS Separator data sheets.

- (Optional) Pre-Separation Filter (# 130-041-407).

## 2. Protocol

### 2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMC should be isolated by density gradient centrifugation (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

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

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

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

### 2.2 Magnetic labeling

▲ Work fast, keep the 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 less 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 $2x10^7$ 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 separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filter # 130-041-407) to remove cell clumps which may clog the column.

▲ Biotinylated primary antibodies should be titrated to determine the optimal labeling dilution. Labeling of the negative population must be avoided.

▲ The concentration of Anti-Biotin MultiSort MicroBeads used to achieve optimal magnetic separation and release of MultiSort MicroBeads is primarily dependent on the intensity of labeling with the biotinylated antibody and, to some degree, also on the frequency of target cells in suspension. Weakly labeled target cells require a higher concentration of Anti-Biotin MultiSort MicroBeads to achieve optimal magnetic labeling and separation. Target cells with a high frequency (>50%) may also require a higher concentration of Anti-Biotin MultiSort MicroBeads than target cells with lower frequencies (see Table 2).

1. Determine cell number.
2. Centrifuge at 300xg for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet.
4. Label cells with biotinylated primary antibody according to the manufacturer's recommendations. For MACS biotinylated antibodies, resuspend $10^7$ total cells in 100 µL buffer and add 10 µL biotinylated antibody.

   ▲ **Note:** If second parameter sorting is performed indirectly, we recommend simultaneous labeling of the cells with the biotinylated primary antibody for the first parameter sorting and the e.g. PE-conjugated antibody for the second parameter sorting.

5. Mix well and incubate for 10 minutes in the dark at 4–8 °C or according to the manufacturer's recommendations.

   ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

6. Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300xg for 10 minutes.
7. (Optional) Repeat washing step.
8. Pipette off supernatant completely.
9. Resuspend cell pellet in buffer and add Anti-Biotin MultiSort MicroBeads according to recommendations in Table 2. For more details see also section 2.5.

**Table 2: Buffer and MicroBead volumes per $10^7$ total cells**

| Antigen Expression | Buffer | Anti-Biotin MultiSort MicroBeads |
|---|---|---|
| "weak" | 80 µL | 20 µL |
| **"intermediate"** | **90 µL** | **10 µL** |
| "strong" | 96 µL | 4 µL |

▲ **Note:** A too strong dilution of the Anti-Biotin MultiSort MicroBeads may result in poor retention of the biotin-labeled target cells. A too low dilution may result in less efficient release of MicroBeads.

10. Mix well and incubate for 15 minutes at 4–8 °C.

    ▲ **Note:** Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.

11. (Optional) Add 10 µL Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC ( # 130-090-856), mix well and incubate for 5 minutes in the dark at 4–8 °C.
12. Wash cells carefully by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300xg for 10 minutes.





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13. Pipette off supernatant completely.
14. Resuspend up to $10^8$ cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
15. 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 the magnetically labeled cells (see Table 1).

**Magnetic separation with MS or LS Columns**

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:
   MS: 500 µL LS: 3 mL
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.
   MS: 3x500 µL LS: 3x3 mL
   Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger, supplied with the column.
   MS: 1 mL LS: 5 mL
   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a second, freshly prepared column.
7. Proceed to removal of MultiSort MicroBeads (2.4).

**Magnetic separation with the autoMACS™ Separator**

▲ Refer to the "autoMACS User Manual" for instructions on how to use the autoMACS™ Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose a positive selection program according to the recommendations in the "autoMACS User Manual".
   ▲ **Note:** Program choice depends on the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".
3. Proceed to removal of MultiSort MicroBeads (2.4).

### 2.4 Removal of MultiSort MicroBeads using MultiSort Release Reagent

1. Remove a sample to analyse the separation by flow cytometry and proceed with remaining magnetically labeled fraction.
2. Add 20 µL MultiSort Release Reagent per 1 mL cell suspension
3. Mix well and incubate for 10 minutes at 4–8 °C.
4. (Optional) To remove any residual magnetically labeled cells, repeat the magnetic separation procedure as described in 2.3. Separate cells over a new column of the same type (MS or LS Columns) or use the same autoMACS program as will be used for the second separation. Collect magnetic (non-released) and non-magnetic (released) cell fractions to determine the efficiency of the release reaction (see 2.5).
   ▲ **Note:** This step is extremely important if the target cells of the second parameter sorting are rare (<10 % target cells in the positive fraction after first sort).
5. Wash cells from the released fraction carefully by adding 1–2 mL of buffer per $10^7$ cells and centrifuge at 300xg for 10 minutes.
6. Pipette off supernatant completely.
7. Resuspend cells in buffer in a final volume of 50 µL per $10^7$ total cells.
8. Add 30 µL of MultiSort Stop Reagent per $10^7$ total cells and mix well.
9. Add the recommended amount of direct or indirect MACS MicroBeads (see "MicroBead data sheets") to magnetically label the cells for the second marker. Adjust to 100 µL total volume by adding buffer.
   ▲ **Note:** The Anti-Biotin antibody is of mouse IgG1 isotype. Thus, Goat Anti-Mouse IgG MicroBeads or Rat Anti-Mouse IgG1 MicroBeads cannot be used for second parameter sorting. When using other Anti-Immunoglobulin MicroBeads for the second parameter sorting, any reactivity with the isotype of the biotinylated primary antibody of the first parameter sorting has to be excluded.
10. Mix well and incubate for 15 minutes at 4–8 °C.
11. Continue as described in the "MicroBead data sheet" and proceed to magnetic separation.

### 2.5 Recommendations to optimize cell isolation using Anti-Biotin MultiSort Kit

The efficiency of the release of the MultiSort MicroBeads is dependent on the strength of the magnetic labeling with Anti-Biotin MultiSort MicroBeads. The strength of the magnetic labeling depends on the intensity of labeling with the biotinylated primary antibody and on the amount of Anti-Biotin MultiSort MicroBeads used for magnetic labeling (see Table 2). Typically, a release > 90% is obtained when working with the Anti-Biotin MultiSort MicroBeads.

$$\textbf{Release (\%)}: \frac{100 \times (\text{No. of cells in released fraction})}{(\text{No. of cells in released + non-released fractions})}$$

Too strong magnetic labeling due to too high a concentration of the Anti-Biotin MultiSort MicroBeads results in an insufficient release of the Anti-Biotin MultiSort MicroBeads. An insufficient release decreases the purity of the cells after positive selection according to the second parameter.

- Use a lower amount of Anti-Biotin MultiSort MicroBeads if the recovery of the target cells after the primary parameter sorting with the Anti-Biotin MultiSort MicroBeads is sufficient but the release is < 90%.

Too weak magnetic labeling due to too low a concentration of the Anti-Biotin MultiSort MicroBeads results in low recovery of the biotin-labeled target cells in the positive fraction.

- Use more Anti-Biotin MultiSort MicroBeads if the release is efficient (> 90%) but the recovery of the positive cells after the first positive selection with the Anti-Biotin MultiSort MicroBeads is too low.





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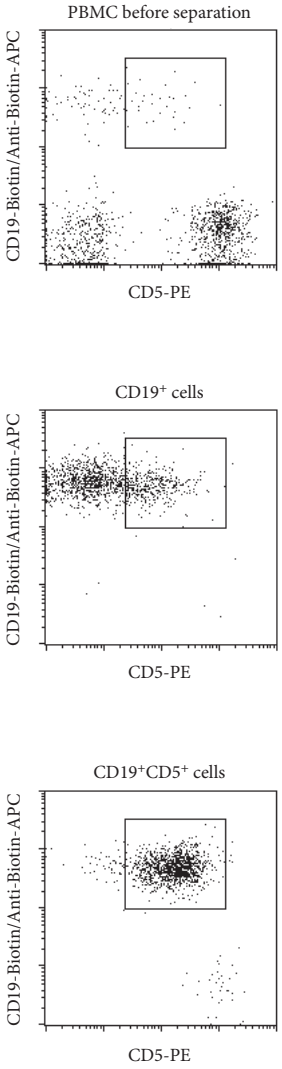
## 3. Example of a separation using Anti-Biotin MultiSort Kit

Separation of human peripheral blood $CD19^+CD5^+$ cells using CD19-Biotin, Anti-Biotin MultiSort Kit and an LS Column for the first positive selection and CD5-PE and Anti-PE MicroBeads and an MS Column for the second positive selection.




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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