

With MACS® Technology hybridoma cells which express antibody on the cell surface can be positively selected. For MACS separation, cells are magnetically labeled with Anti-Immunoglobulin MicroBeads and separated on a MACS Column which is placed in the magnetic field of the MACS Separator. The magnetically labeled antibody producing cells are retained in the column and separated from the unlabeled non-producing cells. After removal of the column from the magnetic field, the magnetically retained antibody producing hybridoma cells can be eluted as positively selected cell fraction.

Instrument and reagent requirements

- MiniMACS™ Separator, VarioMACS™ Separator or SuperMACS™ Separator; MS Columns; MS Column Adapter in combination with VarioMACS Separator or SuperMACS Separator; Column Adapter for MS, LS and LD Columns in combination with SuperMACS™ II Separator.
- Rat Anti-Mouse IgG1 MicroBeads (# 130-047-102), Rat Anti-Mouse IgG2a+b MicroBeads (# 130-047-202) or Rat Anti-Mouse IgM MicroBeads (# 130-047-302).
- Buffer: phosphate buffered saline supplemented (PBS) with 0.5% bovine serum albumin (BSA) and 2 mM EDTA, pH 7.2.
- Fluorochrome conjugated anti-isotype antibody.

Protocol for the positive selection of hybridoma cells

Magnetic labeling

- Determine the number of antibody producing hybridoma cells by staining of the intracellular antibodies with fluorochrom-conjugated anti isotype antibody.
- Centrifuge a volume of cell suspension that contains 10^7 antibody producing cells at $300\times g$ for 10 minutes.
- Carefully remove supernatant completely and resuspend cell pellet in PBS containing BSA (total volume of 500 μ L).
- Add 500 μ L Anti-Immunoglobulin MicroBeads (Rat Anti-Mouse IgG1, Rat Anti-Mouse IgG2a+b or Rat Anti-Mouse IgM), mix well and incubate for 45–60 minutes at 4–8 °C.
- Mix the cell suspension during the incubation time.
- Add 10 mL of PBS containing 0.5% BSA and centrifuge at $300\times g$ for 10 minutes.
- Carefully remove supernatant completely and resuspend cell pellet in 500 μ L of PBS containing BSA.

Magnetic separation

- Place an MS Column (combined with the appropriate Column Adapter) in the magnetic field of the MACS Separator.
- Prepare column by washing with 500 μ L of buffer (for details, see "Column data sheets").

- Apply cell suspension onto the column. Let the unlabeled cells pass through. Rinse with 6×500 μ L of buffer.
- Remove column from separator, place column on a suitable collection tube, pipette 1 mL of buffer onto the column and flush out antibody producing cells using the plunger supplied with the column.
- Count cells of the magnetically labeled fraction and take cells into culture with $1-5\times 10^5$ cells per mL medium.

Evaluation of the enrichment

Determine the frequency of antibody producing hybridoma cells after expansion by staining intracellular antibodies with fluorochrome-conjugated anti isotype antibody. Compare it to the frequency of producing hybridoma cells in the starting sample.

Note

- ▲ The procedure can be repeated after expansion of the positively selected cells to increase the number of antibody producing hybridoma cells.

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