Positive selection of antibody expressing hybridoma cells

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×g for 10 minutes.
- Carefully remove supernatant completely and resuspend cell pellet in PBS containing BSA (total volume of 500 µL).
- Add 500 µL 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×g for 10 minutes.
- Carefully remove supernatant completely and resuspend cell pellet in 500 µL 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 µL of buffer (for details, see "Column data sheets").
- Apply cell suspension onto the column. Let the unlabeled cells pass through. Rinse with 6×500 µL 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 1–5×10^5 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.

SP91.0007

Miltenyi Biotec GmbH
Friedrich-Ebert-Str. 68
51429 Bergisch Gladbach, Germany
Phone +49-2204-8306-0 Fax +49-2204-85197

Miltenyi Biotec Inc.
12740 Earhart Avenue, Auburn CA 95602, USA
Phone 800 FOR MACS, 530 888-8871
Fax 530 888-8925