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Customer protocol

Purification of YSD antibody libraries using the autoMACS® Pro Separator

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Background

Developing antibodies using yeast surface display (YSD) from immunized mice is a modular process. Immune libraries generated from mice typically consist of 10^6 clones and must be enriched for antigen binders. Selecting clones from these libraries requires stringent selection procedures. In this report, we provide a protocol for the rapid and efficient enrichment of antigen binding clones from an immune library by utilizing MACS® Technology and the autoMACS® Pro Separator.

Separation of yeast cells using MACS® Technology and the autoMACS® Pro Separator

A library of single-chain variable fragments (scFv), derived from the genome of B cells from immunized mice, is displayed on the surface of yeast cells. Using MACS® Technology, the scFv are screened and selected for antigen-binding capacity. Hence, yeast cells are first incubated with the biotinylated antigen. Subsequently, antigen-binding yeast cells are magnetically labeled with biotin-binding µMACS™ Streptavidin MicroBeads. During automated separation with the autoMACS® Pro Separator, the sample is applied to an autoMACS Column placed in the magnetic field of the instrument. During washing procedures, unlabeled yeast cells pass through the column as the negative flow-through fraction, while magnetically labeled yeast cells are retained on the column.
After retrieving the magnet from the column, magnetically labeled yeast cells are eluted as positive fraction.
An overview of using MACS Technology in YSD is presented in figure 1.

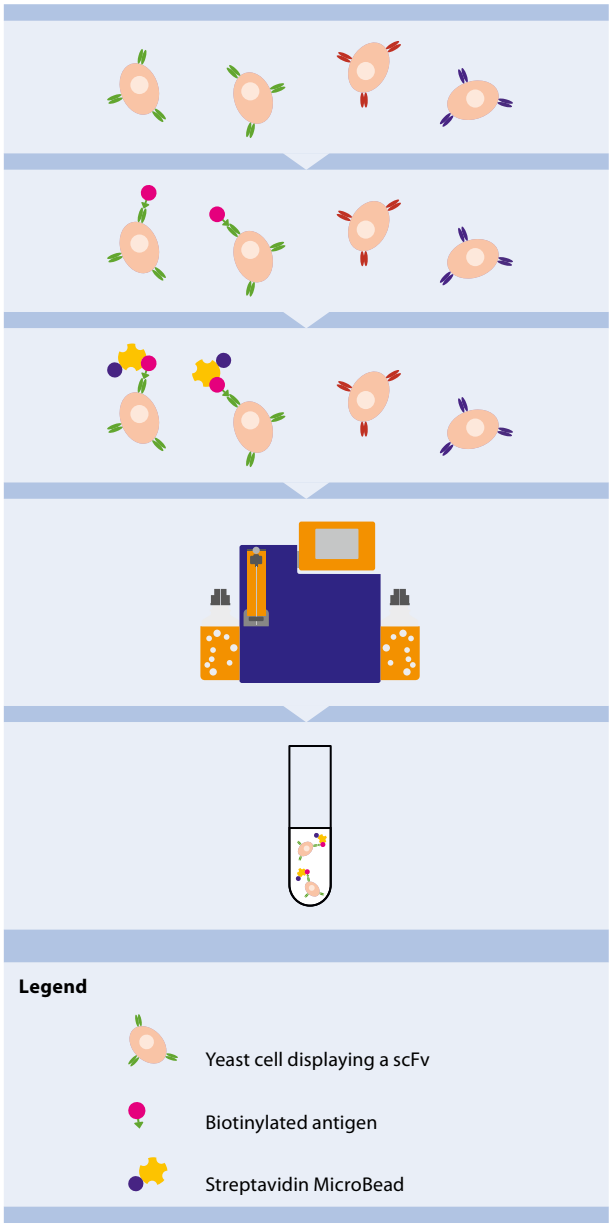

Figure 1: Overview of the purification of YSD antibody libraries using MACS Technology and the autoMACS Pro Separator.

Materials and methods

The establishment of an immune library for YSD from antigen-specific B cells of immunized mice, its growth and induction as well as the archiving of clones is described in detail elsewhere.[1]

Materials

- YSD library from antigen-specific B cells of immunized mice[1]
- biotinylated antigen
- µMACS™ Streptavidin Kit (# 130-074-101)
- autoMACS® Pro Separator
- Chill 50 Rack (# 130-092-953)
- wash buffer[1]
- SD-CAA medium[1]
- SD-CAA plates[1]

Methods

Labeling of cells

1. Aliquot 1×10^{10} scFv expressing yeast cells in a 50 mL tube and centrifuge at 2500×g for 5 minutes at 25 °C. Discard the supernatant.
2. Wash cells with 50 mL wash buffer. After centrifugation, discard the supernatant.
3. Resuspend the cell pellet in 25 mL wash buffer containing the biotinylated antigen.

Note: For the selection of high-affinity clones from an immune library it is recommended to use 0.1 to 10 nM of the antigen. However, in case of low yields, higher antigen concentrations can be used.

4. Incubate the sample with gentle agitation at room temperature for 30 minutes.
5. After incubation, place the sample on ice for 10 minutes.

Note: In order to prevent antigen dissociation from the scFV, all subsequent steps should be performed on ice and with ice-cold solutions.

Cell separation using the autoMACS® Pro Separator

Note: The capacity of the autoMACS® Column is 5×10^{9} cells. Therefore, split the sample into two new 50 mL tubes. Incubate one sample on ice while processing the other one.

6. Centrifuge cells at 2500×g for 5 minutes at 4 °C. Discard the supernatant.
7. Wash cells twice with 25 mL ice-cold wash buffer.
8. For magnetic labeling, resuspend the cell pellet in 2.5 mL ice-cold wash buffer containing 15 µL µMACS Streptavidin MicroBeads and incubate with gentle agitation at 4 °C for 10 minutes.

Note: If this is a second round of magnetic separation, using Anti-Biotin MicroBeads (# 130-090-485) instead of Streptavidin MicroBeads is recommended. The use of alternate MicroBeads will reduce the possibility of selecting scFv specific for streptavidin or anti-biotin antibodies, respectively. Using Anti-Biotin MicroBeads will prolongate the incubation to 30 minutes.

9. After incubation, add 22.5 mL ice-cold wash buffer to the sample and mix gently.

Note: The sample should be mixed gently directly before it is subjected to cell separation to avoid sedimentation of cells.

10. Place the sample in position A1 of a pre-cooled Chill 50 Rack. Place an empty 50 mL and 15 mL tube for the collection of the negative and positive fraction in positions B1 (negative fraction) and C1 (positive fraction) of the rack, respectively. Place the rack onto the MiniSampler of the autoMACS Pro Separator.
11. Go to the **Separation** menu of the autoMACS Pro Separator. Select **position 1** in the sample rack template field.
12. Select "/" from the **Labeling** submenu for manual labeling.
13. Enter the sample volume in the **Volume** submenu. Select **Enter**.
14. Select the program **Posseld** from the **Separation** drop-down menu and a wash program from the **Wash** drop-down menu.
15. Select **Ok** and **Run**.
16. Repeat steps 6 to 15 for the second sample.
17. Rinse the 50 mL tubes holding the original samples with ice-cold wash buffer and subject those cells to magnetic separation by repeating steps 6 to 15.
18. Combine all positive fractions and measure the OD600 of the sample.
19. Centrifuge at 2500×g for 5 minutes at 4 °C. Discard the supernatant. If the OD600 was below 0.5, resuspend the cell pellet in 0.5 mL SD-CAA medium. Otherwise resuspend the cell pellet in SD-CAA medium to a final OD600 of 0.5.
20. To confirm the size of the selected library, plate a dilution of the sample on SD-CAA plates. Usually, the yield for the first round of magnetic cell separation is between 10^{5} to 10^{6} cells.

Results

A yeast surface display library of scFv created from mice immunized with prostate-specific antigen (PSA) was enriched for PSA binders using biotinylated PSA and µMACS Streptavidin MicroBeads. The yeast clones displaying scFv that bind PSA were enriched from 0.17% in the starting library to 44% following one round of enrichment using the autoMacs Pro Separator. The bivariate plots of the cytometry data are shown in figure 2.

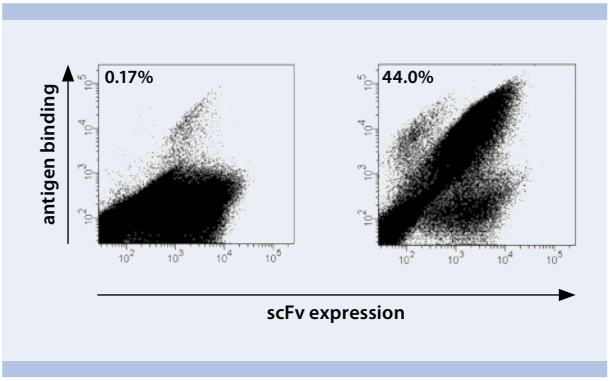

Figure 2: Dot plots of yeast cells stained for full lenght scFv and PSA binding. A yeast surface display library of scFv generated from mice immunized with prostate-specific antigen (PSA) was enriched for PSA binders using biotinylated PSA and µMACS Streptavidin MicroBeads. Briefly, 10^{10} yeast cells from the library (estimated size: 10^6) were incubated with 1 nM biotinylated PSA, washed to remove unbound antigen, incubated with µMACS Streptavidin MicroBeads, and separated on the autoMACS Pro Separator using the program "possel". Approximately 5×10^6 yeast cells were eluted as positive fraction from the column. The unselected and selected libraries were analyzed by flow cytometry. For the cytometric analysis, cells were stained with 1 nM biotinylated PSA and PE conjugated streptavidin and an Alexa Fluor® 488 labeled antibody that recognizes the c-Myc tag on full length scFv clones.

Cheryl L. Baird:
"My group uses directed molecular evolution and yeast surface display to develop affinity reagents. We've been using the Miltenyi autoMACS system for over 4 years to perform our first rounds of enrichment when we are working with large yeast libraries.[1] The system is simple, fast, and orders of magnitude better than doing magnetic bead enrichments by hand! Miltenyi customer support is excellent, too!"

References:

1. Miller K. D. *et al.* (2008) Curr. Protoc. Cytom. Chapter 4: Unit 4.7. Review.

Visit **www.automacspro.com** for more information on the autoMACS Pro Separator or find more about MACS Cell Separation Reagents at **www.macscellseparation.com**