Materials and Equipment

- Pro5™ recombinant MHC Pentamer, biotin-labeled.
- Streptavidin magnetic beads to detect the biotin label of the Pentamer, suitable for use with magnetic columns (e.g. Streptavidin Microbeads, Miltenyi Biotec #130-048-102)
- Streptavidin, conjugated to fluorescent label of choice
- Anti-CD8 antibody, conjugated with a different fluorescent label to streptavidin
- Wash buffer, de-gassed (0.1% sodium azide, 0.1% BSA in PBS)
- Fix solution (1% fetal calf serum, 2.5% formaldehyde in PBS)
- Magnetic stand e.g. MACS Multistand, Miltenyi Biotec #130-042-303
- Separation unit e.g. MiniMACS separation unit, Miltenyi Biotec #130-042-102
- Magnetic columns e.g. MS Columns, Miltenyi Biotec #130-042-201
- Benchtop refrigerated centrifuge with swing-out rotor and appropriate carriers

Standard Procedure *(may need adjustment depending on the particular system used)*

Procedure for washing cells

Dispense 1 ml wash buffer per tube and spin 400 × *g* for 5 minutes in a chilled centrifuge at 4ºC. Check for presence of a cell pellet before discarding the supernatant. Resuspend cell pellets in residual liquid (~50 µl).

1. **Stain 1 × 10^7 cells with 5 tests biotin-labeled Pentamer for 10 minutes at room temperature.**
2. **Wash samples, centrifuge and discard the supernatant.**
3. **Resuspend cells in 90 µl wash buffer and add 10 µl streptavidin magnetic beads per tube** (or as directed by the manufacturer).
4. **Incubate for 15 minutes at manufacturers recommended temperature.**
5. **Wash cell-bead complexes, centrifuge and resuspend in 500 µl wash buffer (de-gassed).**
6. **Meanwhile, wash a column suitable for positive-selection with 500 µl wash buffer (de-gassed) and place on magnetic stand.**
7. **Load cell-bead complexes onto the column.** Antigen-specific T cells labeled with Pentamer-bead complexes will be retained on the column (positive fraction).
8. **Collect the negative fraction that elutes from the column, including 3 washes of 500 µl each.**
9. **Remove column from magnet and flush out the positive fraction by adding 1 ml wash buffer onto the column and applying a plunger (provided with the column).**
10. **To obtain a purer antigen-specific cell population, the positive cell fraction may be passed over a second column.**
11. **Stain cells from pre-isolation, positive and negative fractions with anti-CD8 antibody and fluorescent streptavidin for flow cytometric analysis.**

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