

Materials and Equipment

- Pro5™ Recombinant MHC Pentamer, conjugated to fluorescent label of choice.
- Anti-fluorochrome magnetic beads, e.g. anti-R-PE or anti-APC (Miltenyi Biotec / BD Biosciences), to detect the fluorescence of the Pro5™ Pentamer, suitable for use with magnetic columns.
- Anti-CD8 antibody, conjugated with a different fluorescent label to the Pro5™ Pentamer
- Wash buffer (0.1% sodium azide, 0.1% BSA in PBS) - remove air prior to use (de-gassed)
- 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 $400 \times g$ for 5 minutes in a chilled centrifuge at 4°C. 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 50ml (5 tests) fluorescent-labeled Pentamer for 10 minutes at room temperature (22°C).**
- 2. Wash samples, centrifuge and discard the supernatant.**
- 3. Resuspend cells in 80ml wash buffer and add 20ml anti-fluorochrome 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 500ml wash buffer (de-gassed).**
- 6. Meanwhile, wash a column suitable for positive-selection with 500ml 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 column washes of 500ml each.**
- 9. Remove column from magnet and flush out the positive fraction by adding 1ml 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 for flow cytometric analysis.**