Materials and Equipment

- Pro5™ recombinant MHC Pentamer, biotin-labeled.
- Streptavidin magnetic beads to detect the biotin label of the Pentamer, suitable for use with an external magnet (e.g. Dynabeads® M-280 Streptavidin, Invitrogen #112-05D; Lodestars™ 2.7 Streptavidin, Polymer Laboratories #6727-1001)
- 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 tube holder
- 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. **For best results start with at least 1 × 10^7 lymphoid cells (PBMC or splenocytes).**
2. **Wash cells with wash buffer and resuspend in 200 µl wash buffer.**
3. **Add 1 test (10 µl) biotin-labeled Pentamer per 2 × 10^6 cells**
4. **Incubate at room temperature (22ºC for 10 minutes.**
5. **Wash the cells and resuspend in 500 µl wash buffer.**
6. **Add an optimally titrated amount of streptavidin beads.** 5 beads per cell is recommended.
7. **Incubate on ice for 30 minutes with mixing.**
8. **Bring the volume in the tube up to 2 ml with wash buffer then place in a magnetic tube holder.**
9. **Leave for 3-5 minutes.** If desired, supernatant can be retained for flow cytometric analysis to confirm removal of antigen-specific cells, otherwise discard supernatant.
10. **Wash the fraction containing bead-cell complexes 3 times with wash buffer and discard supernatant.**

    Isolated bead-cell complexes may be placed in cell culture, where beads should dissociate after a few days.

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