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 × 10⁷ 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 × 10⁶ 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 35 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.