CIP Protocol for HLA-Multimer staining

Staining procedure
Note that the buffers, incubation times and concentration of HLA-multimers and Ab should be optimized by each lab. Companies providing multimer reagents are generally proposing a protocol which should be followed.
Based on individual experience and results of previous CIP panels, we propose a staining protocol for combined HLA-multimers/Ab.

Materials:

- 96 U well microplates and lids, non-sterile (Greiner bio-one cat n° 650101 and 656101)
- Eppendorf tubes 1.5 ml, non-sterile (Eppendorf 3810X)
- PBS 1X without Ca/Mg (PAA H15-002)
- EDTA stock solution 0.5M (Roth 8040-2)
- Fetal Bovine Serum (PAA A15.102) heat-inactivated
- Washing buffer: PBS 1X + 2% FBS + 2mM EDTA + 0.02% azide
- Tetramer staining buffer: PBS 1X + 50% FBS+ 2mM EDTA + 0.02% azide
- Fix-solution: washing buffer + 1% Formaldehyde solution (Fluka, cat n° 47608)

Method:

- Resuspend the cells at 10-20 x 10^6 / ml in culture medium or washing buffer
- Distribute 100 µl of the cell suspension in 1 well of a U-bottom 96 well plate for each tetramer staining (1-2 Mio cells/ test)
- Wash the cells once by adding 100 µl of washing buffer. Centrifuge the plate 410 g, 5 min 4°C (discard the supernatant by flicking the plate and dry shortly on absorbent paper)
- Prepare multimer at 5 µg /ml in 50 µl staining buffer in a 1.5 ml epi tube; if several tests are to be performed with the same multimer, prepare a master mix. Plan for one test more than needed
- Just before use, centrifuge the multimer solution 14000 g, 5 min, 4°C to eliminate possible aggregates, and transfert immediately the supernatant (-25 µl) containing the multimer in a new epi
- Add 50 µl of the multimer solution/ test on the cells, mix gently by pipetting up and down
- Incubate 30 min at room temperature, in the dark
- Wash once by adding 150 µl of washing buffer / well, mix gently and centrifuge 410 g, 5 min, 4°C, then discard supernatant as described above
- On the pellet, add selected antibodies at pretested dilution (e.g. CD8, CD3). One or several additional Ab can be added for gating out CD8 negative cells, such as CD4, CD19 or CD56 (dump channel). Also a dye for dead cell discrimination can be included. Both procedures have been shown to improve specific detection (not formally addressed by the CIP panels so far). Mix gently
- Incubate 20 min, 4°C, in the dark

- Wash by adding 150 µl washing buffer, mix and centrifuge 410 g, 5 min, 4°C
- Wash by adding 200 µl washing buffer, mix and centrifuge 410 g, 5 min, 4°C
- Resuspend the cells in 250 µl washing buffer and acquire immediately or fix the cells with 200 µl Fix-solution

Date 23 March 2011
C. Gouttefangeas, Department of Immunology, Tübingen