

## F-KLH Staining Procedure

1. Prepare spleen cells and wash 1X in Dulbecco's PBS + 5% FCS (all steps carried out in this medium) (ice).
2. Resuspend 1 spleen/ml medium. Add 3ml/spleen Gey's solution and leave in ice bucket 4 min. Centrifuge cells<sup>AMB AS SUS P 5ND</sup> in ~1ml/spleen and allow to settle at room temperature ~5 min.
3. Then layer cells onto 5ml FCS and centrifuge.
4. Pack a 10 ml plastic syringe loosely with 5 ml unwashed glass wool. Wash through with 10-15 ml medium and allow to come to room temperature.
5. Resuspend cells in 1 ml/spleen and bring them to room temperature by rubbing the tube between the hands. Pass cells into column (2 spleens/column), allow to stand for 2-3 minutes at room temperature, and wash with medium until clear (about 12ml total). Centrifuge.
6. Count (should be ~90% viable) and stain at final cell concentration of  $4-8 \times 10^7$ /ml and final F-KHL concentration of 0.5 mg/ml in D's PBS + 5% FCS 20 min. on ice.
7. Layer cells on top of 1 ml FCS and centrifuge. Resuspend in medium and centrifuge again.