# Bone Marrow (BM) Protocol for Analysing Mast Cells (MC) on the Flow Cytometer[1]

1. Heparinised or EDTA anticoagulated BM aspirations should be used for flow cytometry studies on MCs and specimens should be processed within the first 24 hours after sample collection. In cases in which it is not expected to perform sample processing within the first 24 hours following a BM puncture, a stabilising solution should be used to avoid deterioration of cells e.g. TransFix.
2. BM aspiration should be performed in posterior iliac spina, using an 11 – to 8-G biopsy needle in order to obtain enough BM particles.
3. A minimum of 1.5-2ml BM should be collected and the aspirate passed two or three times through a 25-G gauge needle to disaggregate the BM particles.
4. It is advisable to assess the nucleated cell count at this stage e.g. using a haematology analyser / flow cytometer.
5. **The BM sample should be treated with TransFix[1] (1ml BM + 0.2ml TransFix*) and stored at 2-8°C at this point if it is not to be analysed within 24 hours.**
6. Membrane staining**:** 1.5-2.5 x $10^6$ cells are incubated with previously titrated fluorochrome conjugated monoclonal antibodies (mAb) for 15 mins, room temperature, in the dark.
7. 2ml lysis solution is added to the cells e.g. FACS lysing solution (1/10 in distilled water) and incubated for 10 mins as in step 6.
8. Wash: 4ml PBS is added to the cells and they are centrifuged at 540g for 5 mins, before cells are re-suspended in 500ul PBS.
9. Acquire sample on the flow cytometer.
10. Samples are kept at 4C for a maximum of 24 hours before analysis.
11. Intracellular staining: After membrane staining (step 6), follow wash step 8 except that cells are re-suspended in 100ul of a fixative solution e.g. Solution A, Fix and Perm – An Der Grub and incubated as per step 6.
12. Follow wash step 8 except that cells are re-suspended in 100ul of a permeabilisaton solution e.g. Solution B, Fix and Perm – An Der Grub, together with mAb used to stain cytoplasmic epitopes and incubated as per step 6.
13. Follow steps 8 and 9.

***Cytomark recommends a ratio of 1 part TransFix to 5 parts BM sample however, this ratio may require optimisation to achieve the best possible stabilisation of the sample**

[1] *L. Sánchez-Munoz, C, Teodósio, J.M. Morgado and L. Escribano*
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