A Protocol for Stabilisation of Rat Bone Marrow Mononuclear Cells (BMMC) for Analysis on the Flow Cytometer

1. After culling the subject(s), excise the femurs and tibias for bone marrow collection.
2. Flush the marrow cavities using Dulbecco's modified Eagle's medium (DMEM) to remove the bone marrow.
3. Separate the mononuclear cells using a density gradient such as Ficoll/Paque (GE Healthcare) and wash using either DMEM or PBS.
4. Resuspend the cells in the residual DMEM or PBS and count the viable cells by Trypan Blue exclusion.
5. Dilute the cells with 150µl DMEM or PBS.
6. **Add 30µl* TransFix to the cell suspension and mix by inversion.**
7. Store at 2-8°C for 24 hours prior to immunoassay.
8. Membrane staining**:** Incubate 3 x 10^5 cells with previously titrated fluorochrome conjugated monoclonal antibodies (mAb) for 15-20 mins at room temperature in the dark.
9. Add lysis solution to the cells (according to the manufacturer's instructions) to eliminate and remaining red cells.
10. 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.
11. Acquire sample on the flow cytometer.
12. Stained samples can be kept at 2-8°C for a maximum of 24 hours before analysis.

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

[1] *Hellen J. V. Beiral, Clara Rodrigues-Ferreira, Aline M. Fernandes, Sabrina R. Gonsalez, Nicoli C. Mortari, Christina M. Takiya, Martha M. Sorenson, Cícero Figueiredo-Freitas, Antonio Galina, and Adalberto Vieyra* The Impact of Stem Cells on Electron Fluxes, Proton Translocation and ATP Synthesis in Kidney Mitochondria After Ischemia/Reperfusion. Cell Transplantation, Vol. 23, pp. 207–220, 2014

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Cytomark, a division of Caltag Medsystems Ltd
● Whiteleaf Business Centre ● 11 Little Balmer ● Buckingham ● MK18 1TF ● UK
T: +44 (0) 1280 827460 **F:** +44 (0) 1280 827466 **E:** office@caltagmedsystems.co.uk **W:** www.cytomark.co.uk
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