DNA STAINING USING KRISHAN BUFFER

Adapted from SOP RPCI Department of Flow and Image Cytometry - 2013

REAGENTS:

- **ice cold** 70% ETOH
- FCM Buffer
- DNA (Propidium Iodide) stain in Krishan Buffer – see separate protocol

PROCEDURE:

1. Harvest cells of interest, spin and remove most of the supernatant.
2. Add 2 ml *ice cold* 70% ETOH to cells one drop at a time while vortexing.
3. Incubate at least 30 minutes at 4 ℃.
4. Centrifuge at 3200 rpm at 4 ℃ for 3 minutes.
5. Decant and rack.
6. Add 1 ml FCM buffer to each sample.
7. Centrifuge at 3200 rpm at 4 ℃ for 3 minutes.
8. Decant and rack.
9. Add 1ml DNA stain in Krishan Buffer to each sample.
10. Incubate at least 30 minutes at 4 ℃.
11. Filter the sample through a mesh filter (FACS tube with blue filter cap).
12. Analyze PI fluorescence