Sodium and Potassium Analysis by Flow Cytometry

Preparation of the dyes:

PBFI-AM (Molecular Probes) 50 ug is dissolved in 8.5 ul of DMSO and 8.5 ul of Pluronic F-127. Working concentration 2.5 mM

SBFI-AM (Molecular Probes) 50 ug is dissolved in 8.9 ul of DMSO and 8.9 ul of Pluronic F-127. Working concentration 2.5 mM

Corona Green (Molecular Probes) 50 ug is dissolved in 15.25 ul of DMSO and 15.25 ul of Pluronic F-127. Working concentration of 2.5 mM

Loading of the dyes:

1. Add 2 ul of PBFI-AM or SBFI AM or Corona Green per 1 ml of cells (approximately 5 x 10^5 cells per ml) for a final concentration of 5 uM and vortex gently.

2. Incubate cells are at 37^{o}C for 60 minutes to load the dye.

3. Immediately prior of flow cytometric examination, add 1 ul of propidium iodide (PI; 10 mg/ml stock) for a final concentration of 10 ug/ml.

Flow Cytometric Analysis:

Cells are examined by exciting the PBFI-AM or SBFI-AM dyes with a UV laser.
PI and Corona Green are excited using a 488 nm laser.

A standard optical set-up is used on FACSVantage SE:
PBFI-AM and SBFI-AM are detected using a 424/44 nm filter in front of the UV FL-4 PMT.
PI is detected using a 630/22 nm filter in front of the FL-3 PMT.
Corona Green is detected using a 530/30 in front of the FL-1 PMT.

A standard optical set-up used on the LSR II:
PBFI-AM and SBFI-AM are detected using a 440/40 nm filter in front of the UV-B PMT.
PI is detected using a 695/40 nm filter in front of the 488-B PMT.
Corona Green is detected using a 530/30 in front of the 488-D PMT.

Cells that are PI positive indicating a loss of membrane integrity are eliminated from further ion analysis.