Basic Buffers for Flow Cytometry

Flow Cytometry Staining Buffer (FACS Buffer)

This basic FACS Buffer is a buffered saline solution that can be used for immunofluorescence staining protocols, antibody and cell dilution steps, wash steps required for surface staining and flow cytometric analysis. The buffer contains sodium azide as preservative and animal serum proteins (FBS/BSA) to help minimize non-specific binding of antibodies. The addition of EDTA prevents cell to cell adhesion and clumping.

1x PBS (human or mouse depending on cells)
2-5 % (v/v) FBS (or BSA)
0.5 mM EDTA (increase to 2mM for sticky or adherent cell lines)
2 mM NaN3
Note: NaN3 is added as a preservative. Use the buffer without NaN3 if you want to do functional
assays with bacterial cells.

Permeabilization Buffer

This Buffer can be used in flow cytometry, particularly for permeabilization for intracellular staining procedures.

1x PBS
0.1% (w/v) Saponin
2-5 % (v/v) FBS (or BSA)
2 mM EDTA
2 mM NaN3
Important: Because saponin-mediated cell permeabilization is a reversible process, it is very important to keep the cells in the presence of saponin during intracellular cytokine staining. Perform permeabilization only on fixed cells.

Fixation Buffer

Fixation Buffer can be used in preparation of cells for intracellular staining procedures. The buffer can also be used to preserve light-scattering characteristics and fluorescence stainings of cells that have been stained by immunofluorescence for subsequent flow cytometric analysis.

1x PBS
4 % Paraformaldehyde (PFA)
Important: Fixation with PFA results in reduced forward scatter (size) and tandem dye fluorescent intensities.
Fixation can also alter receptor epitopes, as such it is recommended to surface stain your cells first and then fix afterwards.

Erythrocyte Lysis Buffer (10x)

This Erythrocyte lysis buffer can be used for quick removal of red blood cells from whole blood, tissues and tumor cells with minimal effects on leukocytes. Prepare a fresh 1:10 working solution in deionized water before use.

1.5 M NH_4Cl

100 mM $NaHCO_3$

10 mM EDTA

pH7.4

Important: Filter this buffer through a 0.2 µm filter (do not autoclave).

AnnexinV Binding buffer

10 mM HEPES pH7.4

140 mM NaCl

2.5 mM $CaCl_2$ (Calcium is crucial for binding of AnnexinV to PS)

Note: Incubate your cells with AnnexinV in binding buffer at room temperature (not at 4 °C) in the dark.