

Cell Surface Labeling and 'TUNEL' Protocol

Reagents

Rat anti-Mouse CD4-PE
Rat anti-Mouse CD8-FITC
HBSS+ (HBSS + 1% BSA)
HBSS
Fresh 2% PFA
SA-APC
Biotin-16-dUTP (1nmol/ul)
DH₂O
100% Ethanol

Terminal Transferase – TdT
(Roche Cat. # 220 582)
Kit supplied solutions:
5X Reaction buffer and CoCl₂

Rxn. Mix

33ul dH₂O
10ul 5X Reaction buffer
5ul CoCl₂ (25mM)
1ul Biotin-16-dUTP
49ul per reaction

Procedure

1. Cell surface label 1-2X10⁶ single cells with CD4-PE and CD8-FITC directly conjugate mAbs. Wash 1X in HBSS and pellet cells.
2. Fix cells in 1% paraformaldehyde (250ul HBSS and 250ul PFA). Incubate on ice 15'. Wash 2X with HBSS (2mls) and pellet cells.
3. Fix cells in 70% Ethanol (150ul HBSS and 350ul 100% Ethanol). Incubate on ice for 20'. Wash 2X with HBSS and pellet cells.
4. Add 49ul Rxn. Mix and 1ul TdT enzyme to each cell pellet and vortex. Incubate at 37⁰C, 30'. Wash 2X with HBSS+. The control is prepared without TdT enzyme.
5. Re-suspend cell pellet in 49ul HBSS+ and add 1ul SA-APC and incubate on ice 30'. Wash cells 2X with HBSS+. Wash cells 1X with HBSS.
6. To the cell pellet add 250ul HBSS and 250ul 2% PFA. Analyze by flow cytometry.