

'TUNEL' Assay with BrdU Protocol

Reagents

dH ₂ O	PBS, Ca ⁺⁺ , Mg ⁺⁺ -free
70% (v/v) Ethanol	1% (w/v) paraformaldehyde/PBS
APO-BRDU™ Apoptosis Kit – BD PharMingen Cat.# 6576KK (Trademark of Phoenix Flow Systems, San Diego, CA) contains:	
FITC labeled anti- BrdU mAb	Reaction buffer
PI/RNase staining soln.	Rinse buffer
Negative Control cells	Wash buffer
Positive Control cells	TdT enzyme
Br-dUTP	

DNA Labeling Soln.

TdT Reaction buffer	10.00ul
TdT enzyme	0.75ul
Br-dUTP	8.00ul
dH ₂ O	<u>32.25ul</u>
Total volume	51.00ul

Antibody Soln./1 reaction

FITC labeled anti- BrdU mAb	5.00ul
Rinse buffer	<u>95.00ul</u>
Total volume	100.00ul

Procedure

1. Prepare 1-2X10⁶ single cells in 500ul PBS.
2. Add the cell suspension into 5mls 1% paraformaldehyde and incubate on ice 15'. Pellet cells and remove supernatant.
3. Wash cells 2X in PBS (5mls). Re-suspend cells in 500ul PBS.
4. Add cell suspension to 5mls ice cold 70% Ethanol. Incubate on ice for 30'. (Cells may be stored in 70% Ethanol at -20⁰C for several days.) Pellet cells and wash 2X in wash buffer. Pellet cells.
5. Remove 1ml from each control cell suspension (in 70% Ethanol, positive and negative) and pellet. Wash 2X in Wash buffer. Pellet cells.
6. Re-suspend cells in 50ul DNA Labeling Solution Reaction and incubate at 37⁰C, 60'. Vortex cells every 15' to re-suspend.
7. Wash 2X each tube of cells with 1.0ml Rinse buffer and pellet. Re-suspend cell pellets in 100ul Antibody Solution Reaction. Incubate at room temp., in the dark, 30'. Do not pellet cells.
8. Add 900ul PI/RNase Staining solution and incubate the cells in the dark, at room temp., 30'.
9. Analyze within 3 hours by flow cytometry.