

TUNEL ASSAY

This assay detects apoptosis in cells.

Materials:

1xPBS
Paraformaldehyde
10% Triton X-100
10% sodium citrate
TUNEL-Staining Kit (Roche)

Procedure:

1. Grow cells on slides in 6-well plate.
2. Wash slides twice with PBS
3. Add 0.5 ml fixing solution (4% paraformaldehyde in PBS, freshly prepared).
Incubate at RT for 1 hour.
4. Remove fixing solution and wash slides twice with PBS.
5. Add 0.5 ml permeabilisation solution (0.1% Triton X-100, 0.1% sodium citrate).
Incubate 2 minutes on ice.
6. Wash slides twice with PBS.
7. Add 50µl TUNEL reaction mixture and incubate in the dark at 37C for 1 hour in a humidified incubator, mixing occasionally.
8. Wash slides twice with PBS.
9. Analyze directly under a fluorescent microscope.

Reference: Roche TUNEL Staining Kit User Manual