

Tunel Staining

- Remove slides from -80°C and allow to warm to room temperature (~10 minutes)
- Bake slides overnight at 40°C
- Wash slides 0.1 M Tris pH 8.0 for 10 minutes
- Circle sections with PAP pen
- Incubate in 5 µg/mL Proteinase K (1:3000 in 0.1 M Tris, 0.05M EDTA pH 8.0) for 10 minutes at room temperature
- Wash slides 2 x for 5 minutes each in 0.1 M Tris pH 8.0
- Incubate in TdT reaction mix for 55 minutes at 37°C
 - TdT buffer:
 - 30 µL 1M tris, pH 7.2
 - 100 µL 10mM CoCl₂
 - 100 µL Na Cacodylate
 - 770 µL H₂O
 - 1:100 of B-16-dUTP
 - 1:100 of TdT enzyme 200-500 units/mL
- Wash slides 2 x for 5 minutes each in 0.1 M Tris pH 7.2
- Wash slides 1 x for 10 minutes in PBS
- Incubate in fluorescent conjugated egg white avidin for 60 minutes
 - 1:66.67 4% NDS
 - FITC 1:50, Cy3 1:500, or AMCA 1:50
 - in PBS
- Wash slides 2 x for 20 minutes each in PBS