TUNEL Stain for Apoptosis in Endothelial Cells and Paraffin-Embedded Tissue

Pre-Treatment

Rinse in PBS: 5 minutes

Fix slides in 4% Formaldehyde Solution: 15 minutes

Prepare 20µg/ml Proteinase K solution in PBS

1. Reconstitute vial of powdered Proteinase K in 1ml Proteinase K buffer
2. Dilute 1:500 in PBS (10µl stock in 5ml PBS)

Rinse slides in PBS: 2x for 5 minutes

Incubate slides in 100µl Proteinase K solution: 10 minutes

Rinse slides in PBS: 5 minutes

Fix slides in 4% Formaldehyde Solution: 5 minutes

Rinse slides in PBS: 5 minutes

Positive control: DNase I treatment

1. Reconstitute 24µl of 10X **DNase I buffer** in 216µl water
2. Incubate in 100µl reconstituted **DNase I buffer**: 5 minutes
3. Add 1.2µl DNase I to 120µl **DNase I buffer**
4. Incubate in 100µl DNase I buffer with DNase I: 5 minutes
5. Wash slide extensively in deionized water: 4 x 1 minute with agitation
6. ***From this point forward, treat positive control slide in separate staining jars***

Incubate slides in 100µl Equilibration Buffer: 10 minutes

Treatment (In Darkness)

Prepare rTDT Incubation Buffer

1. Thaw Nucleotide Mix on ice for 5 minutes, then at room temperature for 5 minutes
2. For each slide, mix 45µl Equilibration Buffer + 5µl Nucleotide Mix + 1µl rTDT
3. For negative control slide, create alternate buffer: 45µl Equilibration Buffer + 5µl Nucleotide Mix + 1µl microwaved, deionized water
4. ***From this point forward, treat negative control slide in separate staining jar***

Apply 50µl rTDT solution per slide, add coverslip and incubate slides for 60 minutes in the slide moat set to 37C

Incubate slides in 2X SSC diluted in water: 15 minutes at room temperature

Rinse slides in PBS: 3 x 5 minutes

Mount slides with DAPI Antifade Reagent

Once dry and sealed, store slides in 4C fridge in a dark slide box

Laboratory of Carlo Bartoli, MD, PhD
Prepared by Samson Hennessy-Strahs, BA
Hospital of the University of Pennsylvania