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## In Situ Nick Translation (ISNT) for the Detection of Apoptotic Cells in Skin

1. Make cryo sections of human skin (7 µm thick) and mount them on poly-L-lysine coated cover glasses. Let the sections dry for 30 min at room temperature.
2. Fixation in acetone (precooled) for 10 min at 4°C.
3. Wash in PBS for 5 min at r.t.
4. ISNT-reaction: 100 µl/ cover glass; 40 min at r.t.: amount for 1 ml reaction mixture

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|---|---|
| 3 µM FITC-12-dUTP (Boehringer 1373242): | 3.75 µl (0.8 mM stock solution) |
| (or 3 µM Biotin-16-dUTP) | |
| 3 µM dGTP | 7.5 µl (0.4 mM stock) |
| 3 µM dATP | 7.5 µl (0.4 mM stock) |
| 3 µM dCTP | 7.5 µl (0.4 mM stock) |
| DNA-Polymerase I (Boehringer 642711) | 50 units/ml (10 µl of 5 u/µl stock) |
| (endonuclease-free) | |
| 10x reaction buffer | 100 µl |
| (50 mM Tris/HCl pH7.5, 10 mM $MgCl_2$, 0.1 mM DTT) | |
| A. dest nuclease-free | ad 1 ml |

5. Washing with PBS: 3 times for 5 min at r.t.
6. Protein block: 30 min at r.t. with 10% FCS in PBS
7. Incubation with peroxidase-conjugated anti-FITC (Boehringer; 1:25 in block solution; 30 min at 37°C)
8. Washing with PBS: 3 x 5 min at r.t.
9. Metal-enhanced DAB-staining (Pierce, 34065): 100 µl/cover glass: Incubation about 5 - 20 min (r.t.)

   Mounting and conventional microscopy (maybe after hemalaun counterstaining)

   Alternative: (if Biotin-16-dUTP was used):

7. Incubation with FITC- or Texas Red conjugated streptavidin (Amersham)
8. Washing with PBS: 3 or 4 times for 5 min at r.t.
9. Mounting and fluorescence or laser scanning microscopy