

Immunohistochemistry using the “milk method” with 0.01% Triton X-100

Thanks to Thenaa Said, PhD at Baylor College of Medicine for first working out the conditions for p27 staining using this method.

This method works for the following antibodies: p27, cyclin D1, RbAP46 (See IHC table for dilutions)

- Deparaffinize and rehydrate sections:
 - 3 x 3' Xylene
 - 3 x 2' 100% ethanol
 - 1 x 2' 95%, 80%, 70% ethanol (each)
 - 1 x 5' 1x PBS
 - Optional: Block endogenous peroxidases 15' at room temperature in 3% H₂O₂ (20 ml of 30% H₂O₂ + 180 ml 1X PBS)
 - Wash 3 x 5' in 1x PBS
 - Shake off/wipe off excess PBS and circle all sections with PAP pen.
 - Block 2-5 hours with 5% milk in PBST (0.01% Triton-X100), ~50 µl per section
 - Dilute 1° Ab in 1% milk in PBS (50 µl/section).
 - Incubate overnight at room temp in a humid chamber.

 - Wash slides 3 x 10' in 1x PBS.
 - Dilute biotinylated 2° antibody at 1:250 in buffer (1% milk in PBS).
 - Add 50 ul per section and incubate 1 hour at room temp in a humid chamber.
 - Wash slides 3 x 10' in 1x PBS.
 - Add ABC reagent to sections and incubate samples for 30 minutes at room temp.
 - Wash 3 x 10' in 1X PBS
 - Make DAB according to Vector protocol in ddH₂O. WEAR GLOVES.

 - Add immediately to slides and wait for color change (let go up to 10-15 minutes)
 - Drain slides and put into ddH₂O for 5 minutes. Dispose of DAB waste with bleach.
 - Counterstain with hematoxylin
 - Quick dip in hematoxylin (5-10 quick dips in stain, no more than 10 sec)
 - Rinse with dH₂O
 - Develop in tap water for 5'
 - Dip 8x in acid ethanol (1 ml concentrated HCl in 400 ml 70% ethanol)
 - Rinse 1 x 2' in tap water
 - Rinse 1 x 2' in dH₂O
 - Dehydrate sections 3 x 5' each in 95% and 100% ethanols
 - Clear in xylene 3 x 15' (can leave overnight)
 - Mount with Permount and coverslip.
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PAP pen	Research Products International Corp	#195500
Vectastain Elite ABC Reagent	Vector Laboratories	PK-7100
DAB	Vector Laboratories	SK-4100
Permount (Histological mounting medium)	Fisher Scientific	#SP15-100