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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for an immunofluorescence assay on paraffin slices.

Attachments

SOP_Staining_Paraffi...
187KB

Materials

- Ethanol
- Xylene
- Citric acid buffer (tri-sodium citrate 2,94 g in 1000 ml distilled water, pH adjusted to 6.0 using 1N HCL)
- Coverslips
- Distilled water
- MilliQ
- DAPI (D3571, Invitrogen)
- Prolong Gold (P36934, Invitrogen)
- PAP pen
- PBS
- Washing buffer: 0.05% Tween-20 (8.22184, Merck Group) in PBS
- Blocking buffer: 1% BSA (A3059-50G, Sigma-Aldrich) and 5% normal donkey serum (ab7475, Abcam) in washing buffer

Rehydration

1 - Xylene, 5 min
- Xylene, 5 min
- Xylene, 5 min
- 96% Ethanol, 5 min
- 96% Ethanol, 5 min
- 96% Ethanol, 5 min
- 70% Ethanol, 5 min
- 50% Ethanol, 5 min
- MilliQ/aqua dest, 5 min
- MilliQ/aqua dest, 5 min
- MilliQ/aqua dest, 5 min

Antigen retrieval

2 - Add citric buffer in microwaveable vessel, use sufficient volume to cover the slides by at least a few cm
- Place vessel in microwave
- Warm up until boiling
- Place slides in microwavable vessel
- Place the vessel back in the microwave for 12 min. Use non-sealed vessel to allow for evaporation. Watch out for boiling over
- After 12 min, remove the vessel and run cold tap water into it for 10 min

Staining

3 - Encircle tissue with PAP pen
- Wash for 5 minutes with Washing buffer
- Block for 30 minutes with Blocking buffer
- Incubate with primary Ab in Blocking buffer overnight at 4°C in a humidified chamber
- Wash 3 times with Washing buffer, 5 minutes each
- Block for 10 minutes with Blocking buffer
- Incubate with secondary Ab in Blocking buffer at RT for 60 minutes (in the dark) including 1:1000 DAPI
- Wash 3 times with Washing buffer, 5 minutes each
- Mount cells in Prolong gold and affix coverslip