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NB300-164 Protocol

Immunocytochemistry/Immunofluorescence Protocol for Bestrophin 1 Antibody (NB300-164)

Bestrophin 1 Antibody (E6-6): https://www.novusbio.com/products/bestrophin-1-antibody-e6-6_nb300-164
Immunofluorescence

1. Paraffin slides. Deparaffinize as follows:
a. 2x 5 min in Xylene
b. 2x 5 min in 100% ethanol
c. 2x 5 min in 95% ethanol
d. 1x 5 min in 70% ethanol
e. 1x 5 min or more in PBS

2. Cryosections:
a. air dry for >30 min
b. rehydrate in PBS-CM (PBS + 0.1mM CaCl2 and 1mM MgCl2) + 3% BSA

3. Use pap pen to draw circles around sections
4. Block in PBS-CM + BSA for 30 min at RT
5. Dilute anti-Bestrophin [cat# NB 300-164] in PBS-CM + BSA and incubate at RT for 1 hour or overnight at 4C.
6. Wash the slides with PBS-CM + BSA 5x 5 min
7. Dilute the secondary antibody in PBS-CM + BSA and incubate at RT for >1 hour (if staining nuclei with propidium iodide add saponin to 0.1% and RNAse A at 1:500)
8. Wash 3x 8 min with PBS-CM + BSA and then 1x 5 min with PBS-CM
a. If staining nuclei with DAPI or propidium iodide, dilute into PBS-CM at 1:1000
b. Wash 3x with PBS-CM, if using propidium iodide
c. Proceed directly to step 9, if using DAPI
9. Mount in Flourmount.

**NOTE: Immunofluorescence Considerations
1. Aldehyde fixatives (ie: PFA and formalin) will not work in immunofluorescence with this antibody.
A) Transfected cells on coverslips can be fixed in acetone or methanol, as can tissue.
B) Paraformaldehyde for paraffin sections can be used if the tissue is subject to heat and pressure mediated antigen retrieval [see specific reference 1 on datasheet]
2. To date, endogenous protein in human or pig eyes cannot be detected, even in methanol/acetone fixed sections directly.
3. Immunohistochemistry, using this antibody, has been done using the vector ABC kit, which includes a signal amplification step.