

## NB300-164 Protocol

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

#### Immunofluorescence

1. Paraffin slides and?? 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 CaCl<sub>2</sub> and 1mM MgCl<sub>2</sub>) + 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.