NEUROMICS

Bestrophin Data Sheet

Catalog Number:	MO25024	**Host:**	Mouse
Product Type:	IgG_1 kappa	**Species Reactivity:**	Human; Pig and Primate
Immunogen Sequence:	Partial recombinant protein [KDHMDPYWALENRDEAHS].	**Format:**	0.1 ml Mouse ascites containing 0.1% sodium.

Applications: Immunofluorescence: Assay dependent (See Applications Notes-Protocol).
Western Blot 1:1,000

Dilutions listed as a recommendation. Optimal dilution should be determined by investigator.

Storage: Store frozen. Aliquot as undiluted antisera and immediately place at -20°C. Antisera may have become trapped in top of vial during shipping. Centrifugation of vial is recommended before opening. Stable for at least 6 months at -20°C. Repeated freeze/thaw cycles compromise the integrity of the antiserum.

Application Notes

This antibody does not work in Rat.

Positive Control: Pig RPE (Retinal Pigment Epithelium) whole cell extract. This antibody recognizes a band at ~68 kDa

Western Blot Procedure
1. Run cell lysates** on an SDS-PAGE gel.
2. Transfer the proteins to PVDF.
3. Block the membrane in 1% Carnation instant milk in PBS + 0.1% Tween 20 (with 0.1mM CaCl2 and 1mM MgCl2) for 1 hour at RT.
4. Dilute the antibody to 1:1,000 in 10 ml of fresh blocking buffer and incubate for 1 hour at RT.
5. Wash the membrane with blocking buffer, 3x 5-10 minutes.
6. Dilute the secondary antibody in fresh blocking buffer, as recommended by the secondary vendor and incubate for 1hour at RT.
7. Wash the membrane with blocking buffer, 5x 8 minutes and rinse 1x with PBS (containing 0.1mM CaCl2 and 1mM MgCl2).
8. Detect the protein-antibody complex with alkaline phosphatase, if using NBT/BCIP or with HRP, if using ECL.

**Cell Lysate Preparation
A. Lysates were prepared in lysis buffer [50mM TrisÂ·HCl, pH 8 / 120mM NaCl / 0.5% Nonidet P-40 / 10 ug/ml aprotinin / 10 ug/ml leupeptin / 1mM phenylmethylsulfonyl fluoride / 1mM sodium orthovanadate].
B. Total protein content was determined by bicinchoninic acid assay (Pierce).

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

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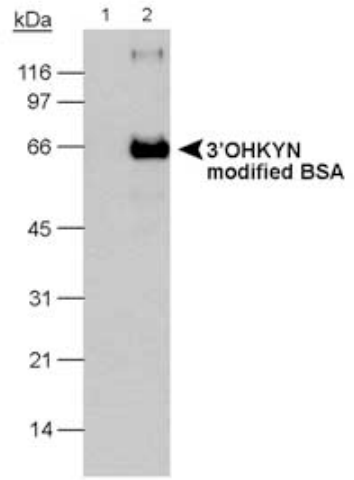
2. Cryosections:
    a. air dry for >30 min
    b. rehydrate in PBS-CM (PBS + 0.1mM CaCl2 and 1mM MgCl2) + 3% BSA
    3. Use pap pen to draw circles around sections
    4. Block in PBS-CM + BSA for 30 min at RT
5. Dilute Bestrophin [cat# NB 300-164] in PBS-CM + BSA and incubate at RT for 1 hour or overnight at 4ºC.
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.

Description/Data:
Best macular dystrophy (BMD) or vitelliform macular dystrophy (VMD2), is an autosomal form of macular degeneration, characterized by a depressed light peak in the electrooculogram (EOG). It is inherited and has an early onset. Bestrophin is a 68 kDa basolateral plasma membrane protein encoded by the VMD2 gene. Bestrophin's function is still unknown, but data suggests that it is a chloride channel that plays a role in generating the altered EOG in Best disease patients. In addition, Bestrophin is a useful biochemical and histological marker of RPE (retinal pigment epithelial cells) cells.



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