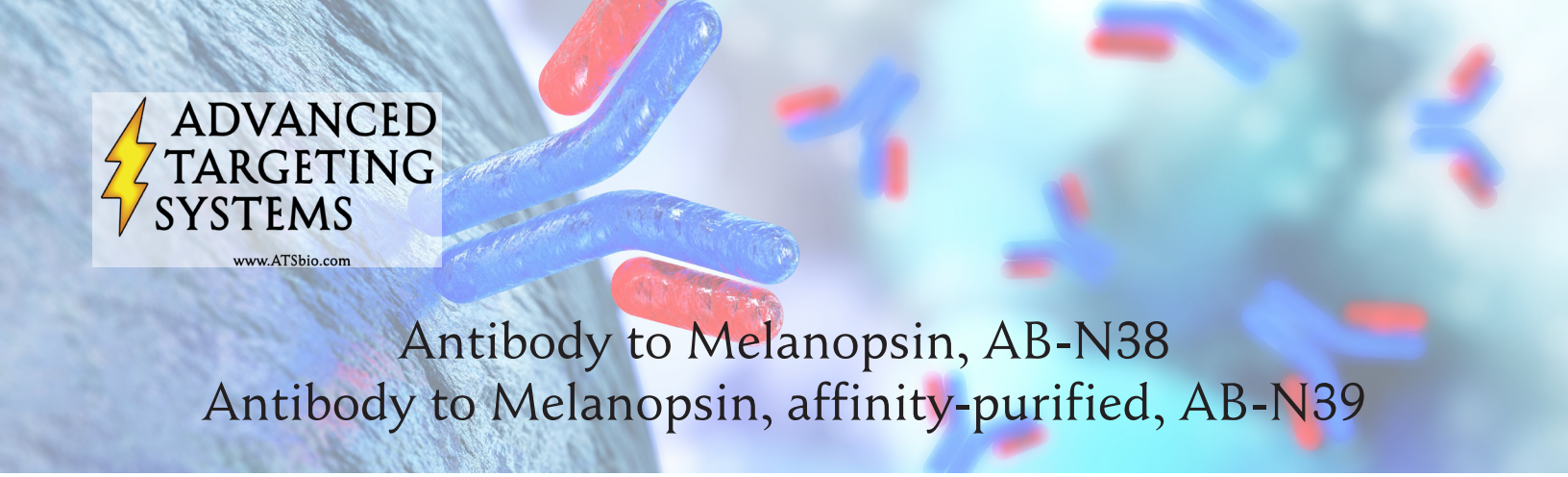

# Antibody to Melanopsin, AB-N38
# Antibody to Melanopsin, affinity-purified, AB-N39

*This is an example protocol. Please follow good laboratory technique and safety guidelines.*
*Working dilutions must be determined for each lot.*
*Please contact us if you have questions. www.ATSbio.com*

## IMMUNOSTAINING PROTOCOL

1. Remove the corneas, and postfix eyes at 4°C for 24 hours in 4% paraformaldehyde in phosphate-buffered saline (PBS). Remove lenses.
2. Cryoprotect eyecups for sectioning at 4°C for 24 hours in 30% sucrose in PBS; embed the eyecups in OCT medium (Sakura Finetek, Torrance, CA), freeze, section (16-20 µm), and thaw-mount onto gelatin-coated slides.
3. Dissect retinas destined for flat-mounting from eyecups immediately after postfixation, stretch onto filter paper, and process in 1.5 ml microfuge tubes.
4. Wash tissue (slides and flat-mounts) 3 times (10 min, 4°C) in Tris- buffered saline (TBS, Quality Biological, Gaithersburg, MD) and block for 30 min at 4°C in 1.5% normal goat serum in TBS.
5. Incubate tissue for 24 hr at 4°C in a 1:2,500 dilution of antiserum UF006 in a TBS-incubating buffer containing 1% bovine serum albumin, 0.25% carrageenan lambda and 0.003% Triton X-100.
6. Wash slides and flat mounts three times in TBS (10 min, 4°C) and incubate for 1 hour at 22°C in Cy3-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) diluted 1:500 in TBS incubating buffer.
7. Wash 3 final times in TBS (10 min, 22°C).
8. Remove flat-mounts from the filter paper and transfer onto glass microscope slides. Mount flat-mounts and sections in DAPI-containing Vectashield (Vector Laboratories, Burlingame, CA), coverslip, and seal with clear fingernail polish.

**Panda S. et al. 2002. Melanopsin (Opn4) requirement for normal light- induced circadian phase shifting Science 298(5601):2213-2216.**