Product no AS12 1851
NpHR | Halorhodopsin

Product information

**Background**  Halorhodopsin (HR) is a hyperpolarizing light-driven ion pump from the halophilic archaea bacterium *Natronomonas pharaonis*. HR uses the energy of yellow light (excitation maximum near 580 nm) to mediate primarily chloride but also bromide, iodide, and nitrate import into the cell against their electrochemical gradients.

**Immunogen**  KLH-conjugated synthetic peptide derived from *Natronomonas pharaonis* halorhodopsin sequences UniProt: A0A1U7EU03

**Host**  Rabbit

**Clonality**  Polyclonal

**Purity**  Affinity purified serum in PBS, pH 7.4

**Format**  Lyophilized

**Quantity**  50 µg

**Reconstitution**  For reconstitution add 50 µl of sterile water.

**Storage**  Store lyophilized/reconstituted at -20°C; once reconstituted make aliquots to avoid repeated freeze-thaw cycles. Please, remember to spin tubes briefly prior to opening them to avoid any losses that might occur from lyophilized material adhering to the cap or sides of the tubes.

**Tested applications**  Immunofluorescence (IF), Western blot (WB)

**Related products**  AS12 1851P | NpHR | Halorhodopsin blocking peptide

Application information

**Recommended dilution**  1 : 200 (IF), 1 : 1000-1 : 20 000 (WB)

**Expected | apparent MW**  30.9 | 30 kDa

**Confirmed reactivity**  *Natronomonas pharaonis*

**Predicted reactivity**  *Natronomonas pharaonis*

**Not reactive in**  *Halobacterium salinarum* (HsHR and HsBR)

**Additional information**  Immunofluorescence was performed on mouse brain slices (data not shown).


Application example
Following SDS-PAGE (12% Separation Gel) the Blot (PVDF) was stained with PonceauS (A, lowest panel), divided in two halves, scanned and blocked with 5 % not-fat milk powder (Marvel) o.n. at 4 °C. The halves were then incubated separately at RT for 1 h with anti-NpHR or pre-immune serum at the indicated dilutions, rinsed briefly twice with TBS-T (pH 7.4), then washed three times for 15 min, incubated for 1 h at RT with goat anti-rabbit-HRP conjugated secondary antibody (Agrisera, # AS09 602) in a dilution of 1:100 000 and washed as described before. The halves were combined for development with TMA-6 (Lumigen) at the indicated exposure times using Kodak X-ray Film (# 8143059)(A). The anti-NpHR blot was stripped at 70 °C for 30 min and re-probed with anti-Myc (Abcam, # ab9106) and anti-rabbit-HRP (Agrisera, # AS09 602, 1:200 000) as described below (B).

Samples loaded in the order as indicated above the blot were 9 µg (12 µg in case of HsHR; Amidoblack Assay) of total membrane fractions of yeast expressing: EV = empty vector control; NpHR = Halorhodopsin from Natronomonas pharaonis; HsHR = Halorhodopsin from Halobacterium salinarum; HsBR = Bacteriorhodopsin (D85T) from Halobacterium salinarum; NpHR, HsHR and HsBR include a C-terminal Myc tag. The calculated molecular weights are NpHR-Myc: 33.6 KDa; HsHR-Myc: 31.4 KDa; HsBR-Myc: 29.4 KDa.

Courtesy of Dr. Annegret Honsbein from Dr. Anna Amtmann's laboratory at the University of Glasgow, United Kingdom.