Anti-Rat NIS antibody

Product Name: Anti-Rat NIS Antibody
Cat. Number: REA008
Lot Number: REA-IM09
Unit Size: 100 µL
Concentration: 1 mg/mL
Storage Buffer: 2M Tris, 0.2M glycine (pH, 7.4)

Isotype: Rabbit polyclonal, Affinity-purified
Reactivity: Rat, Mouse

Product Description

Affinity-purified rabbit polyclonal antibody raised against a synthetic peptide corresponding to residues 603-618 of the rat sodium iodide symporter (rNIS)[1]. This antibody detects both the native and denatured forms of rNIS. This antibody recognizes an intracellular C-terminal epitope that is conserved between rat and mouse NIS.

Storage Instructions

This antibody can be stored short term (1-2 weeks) at 2-8ºC. For longer term storage, aliquot and store at or below -20ºC. Avoid repeated freeze/thaw cycles.

Applications

	Dilution	Notes
Western blotting	1:1,000 - 1:5,000	*see below
Flow cytometry	1:500	†Permeabilization required
Immunofluorescence	1:500	†Permeabilization required
Immunohistochemistry	1:500	†Antigen retrieval required (paraffin)

*For western blotting of NIS proteins, it is recommended to heat samples at 37ºC for 30 minutes (**do not** boil) prior to loading for SDS-PAGE.

†This antibody recognizes the cytosolic C-terminus of rNIS. Therefore, samples must be permeabilized prior to incubation with anti-rat NIS antibody for IHC, IF, and flow cytometry analysis.

Recommended Controls

Rat thyroid FRTL-5 cells (ATCC© #CRL-8305) or cells transduced with lentivirus encoding rat NIS (Imanis #LV007 and LV026) should be used as a positive control.

Recommended Protocol: Protein Extraction

To prepare membrane protein fractions, harvest and homogenize cells at 4ºC in homogenizing buffer (10 mM Tris-HCl, pH7.5, 5 mM NaCl, 1 mM EDTA, 0.25 M sucrose, and 1X protease inhibitor). Clarify lysates at 700 x g for 10 min (4ºC). Centrifuge the recovered supernatant at 200,000 x g for 1 h (4ºC). Resuspend the pellet in homogenizing buffer and store at -70ºC.

To prepare total protein fractions, lyse cells in RIPA buffer containing 1X protease inhibitors. Incubate on ice for 30 min then clarify at 8000 x g for 15 min (4ºC). Store at -70ºC.

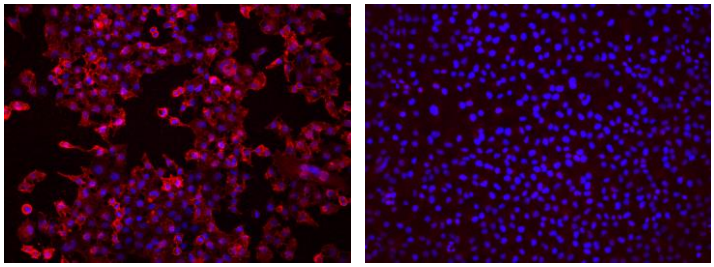
Product Citations:

[1]Levy et al. Proc. Natl. Acad. Sci. USA. 1997. 94:5568-5573
[2]De la Vieja et al. J. Cell Sci. 2004. 117:677-687
[3]Nicola et al. Am. J. Physiol. Cell Physiol. 2009. 296:C654-C662
[4]Tazebay et al. Nat. Med. 2000. 6:871-878
[5]Paroder-Belenitsky et al. Proc. Natl. Acad. Sci. USA. 2011. 108:17933-38.

Certificate of Analysis

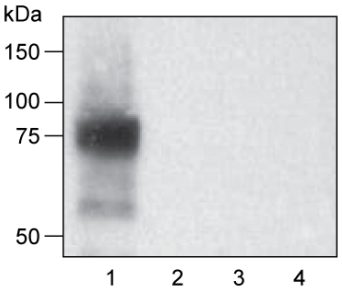
Testing performed by Imanis Life Sciences.

Immunofluorescence



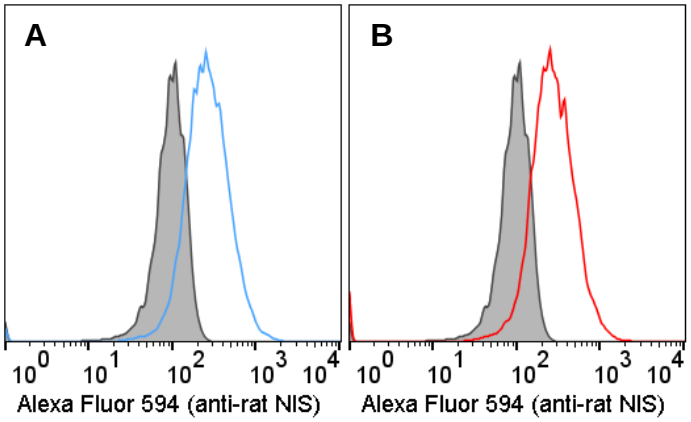
HelaH1 cells stably-expressing (*left*) rat NIS or (*right*) human NIS were permeabilized and stained with anti-rat NIS antibody (1:500 dilution) followed by an Alexa Fluor 594-conjugated anti-rabbit secondary antibody and Hoechst 33342 to stain nuclei. Cell photos were taken at 200X magnification.

Western Blotting



Total protein (10 µg) from HeLaH1 cells stably-expressing rat (lane 1), dog (lane 2), pig (lane 3), or rhesus (lane 4) NIS was subjected to SDS-PAGE and western blot analysis using anti-rat NIS antibody (1:3,000 dilution) and HRP-conjugated anti-rabbit secondary antibody. The top band (~75-90 kDa) represents the hyperglycosylated form of rNIS, while the bottom band (~60 kDa) represents the hypoglycosylated form of rNIS.

Flow cytometry



(A) HelaH1 cells stably-expressing rat NIS (blue) or parental HelaH1 cells (grey) were fixed with paraformaldehyde, permeabilized, and stained with anti-rat NIS antibody (1:500 dilution) followed by an Alexa Fluor 594-conjugated anti-rabbit secondary antibody. Stained cells were subjected to flow cytometry analysis.[5] **(B)** HelaH1 cells stably-expressing mouse NIS (red) or parental HelaH1 cells (grey) were stained with anti-rat NIS antibody (1:500) followed by secondary antibody and analyzed by flow cytometry as described for panel A.

Quality Control by: JM
Quality Assurance by: LS
Effective Date: 10 OCT 2016

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