Anti-Human ETNL NIS Antibody

Product Name: Anti-Human ETNL NIS Antibody
Cat. Number: REA009
Lot Number: REA-IM10
Unit Size: 100 µL
Concentration: 1.5 mg/mL
Storage Buffer: 2M Tris, 0.2M glycine (pH, 7.4)

Isotype: Rabbit polyclonal, Affinity-purified
Reactivity: Human Sodium iodide symporter

Product Description

Affinity-purified rabbit polyclonal antibody raised against a synthetic peptide GHDGGRDQQETNL corresponding to residues 631-643 of the human sodium iodide symporter (hNIS)[1]. Detects both the native and denatured forms of hNIS. This antibody recognizes an intracellular C-terminal epitope. This antibody does not detect rhesus, mouse, pig or dog 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[2, 3]	1:1,000 - 1:5,000	*see below
Flow cytometry[3,4]	1:500	†Permeabilization required
Immunofluorescence[5, 6]	1:500	†Permeabilization required
Immunohistochemistry[1,5]	1:5,000	†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 hNIS. Therefore, samples must be permeabilized prior to incubation with anti-human ETNL NIS antibody for IHC, IF, and flow cytometry.

Recommended Controls

Cells expressing human NIS should be used as a positive control. For western blotting, lysates should be prepared from cells transduced with lentivirus encoding human NIS (Imanis #LV001 or LV002) or stably expressing high-levels of human NIS (Imanis #CL001). Normal human thyroid tissue can be used as a positive control for immunohistochemistry.

Recommended Protocol: Protein Extraction

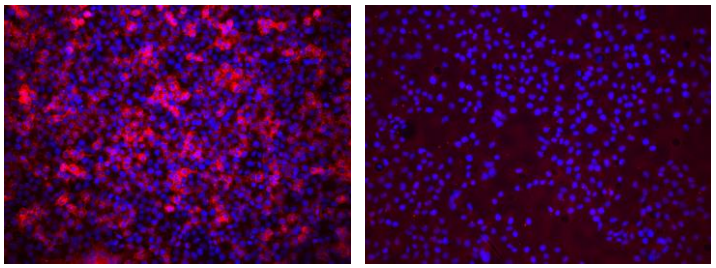
To prepare membrane protein fractions, harvest and homogenize cells at 4ºC in homogenizing buffer (10 mM Tris-HCl, pH7.5, 5 mM NaCl, 1 mM EDTA, 0.25 M sucrose, and 1X protease inhibitor). Clarify lysates at 700 x g for 10 min (4ºC). Centrifuge the recovered supernatant at 200,000 x g for 1 h (4ºC). Resuspend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

Certificate of Analysis

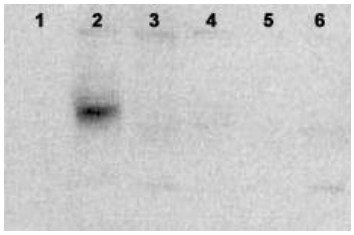
Testing performed by Imanis Life Sciences

Immunofluorescence



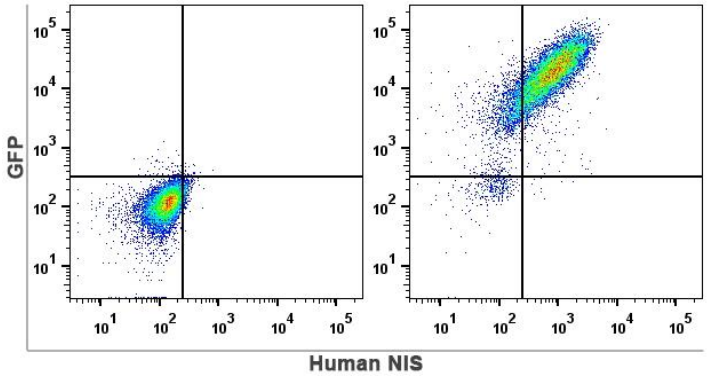
HelaH1 cells stably-expressing (*left*) human NIS or (*right*) rhesus NIS were fixed, permeabilized, and stained with anti-human ETNL NIS antibody (1:500 dilution) followed by an Alexa Fluor 594-conjugated anti-rabbit secondary antibody and Hoechst 33342 to stain nuclei. Cell photos were taken at 200X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

Western Blotting



Total protein (20 µg) from parental HeLaH1 (1) HeLaH1 expressing human (2), mouse (3), rhesus (4), pig (5) and dog (6) NIS were subjected to SDS-PAGE and western blot analysis using anti-Human ETNL NIS antibody (1:3,000 dilution) and HRP-conjugated anti-rabbit secondary antibody. A 90 kDa band of NIS was detected.

Flow cytometry



(*Left*) Parental HelaH1 cells or (*right*) HelaH1 cells stably-expressing human NIS linked to enhanced green fluorescent protein (GFP) were fixed, permeabilized, and stained with anti-Human ETNL NIS antibody (1:500 dilution) followed by an Alexa Fluor 594-conjugated anti-rabbit secondary antibody. Samples were subjected to flow cytometry analysis.

Product Citations:

[1]Wapnir et al. J.Clin Endocrinol Metab. 2003. 88:1880-1888
[2]Li et al. FASEB J 2003. 27:3229-3238
[3]Paroder et al. 2013. J Cell Sci 126:3305-3313
[4]Dohan et al. Mol Endocrinol 2006. 20:1121-1137
[5]De la Vieja et al. 2004. J Cell Sci 117:677-687
[6]De la Vieja et al. 2005. Mol Endocrinol 19:2847-2858
[7]Tazebay et al. 2000. Nat Med 6:871-878

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