# Anti-Human KELE NIS Antibody

**Product Name**: Anti-Human KELE NIS Antibody
**Cat. Number**: REA0010
**Lot Number**: REA-IM11
**Unit Size**: 100 µL
**Concentration**: 0.4 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 KELEGAGSWTPCVGHD corresponding to residues 618-633 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 mouse or rat 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] | 1:1,000 - 1:5,000 | *see below |
| Immunofluorescence[2] | 1:500 | †Permeabilization required |
| Immunohistochemistry[3, 4] | 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 KELE NIS antibody for IHC and IF.

## 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.

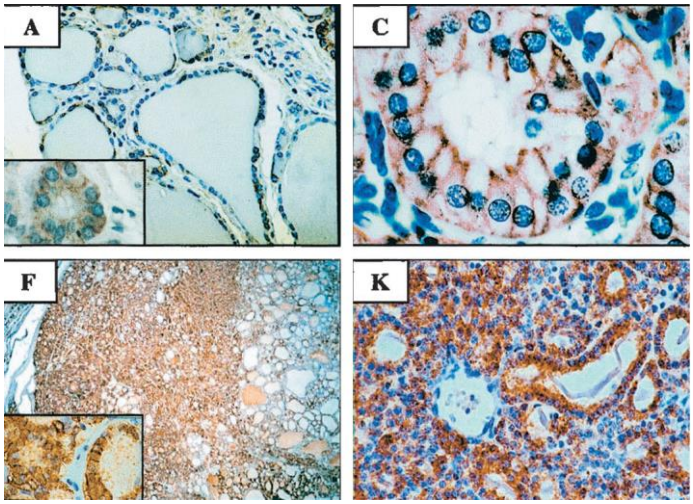
## Product Citations:


[1]Carrasco et al. Methods Enzymol. 1986. 125:453-467
[2]Dohan et al. Mol Endoclinol. 2006. 20:1121-1137
[3]Dohan et al. J Clin Endocrinol Metab. 2001. 86:2697-270
[4]Tazebay et al. Nat. Med. 2000. 6(8):871-8


## Certificate of Analysis

### Immunohistochemistry

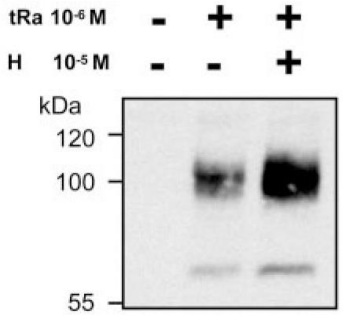
Detection of human NIS by immunohistochecmistry was performed by Dohan et al. 2001.



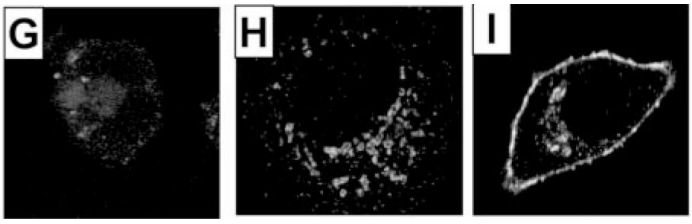

Thyroid tissue sections were deparafinized. Slides were subjected to antigen retreival using 10% citrate buffer. Slides were incubated with 1µg/mL anti-human NIS antibody for 1h. All slides were counterstained with haematoxylin. A. normal thyroid, C. Graves' tissue, F. papillary carcinoma, K. follicular carcinoma.

### Western blotting and Immunofluorescence

Stimulation of NIS protein expression and plasma membrane targeting by all-trans-retinoic acid (tRa) and hydrocortisone was determined by Dohan et al. 2006.




MCF-7 cells were treated for 48h with trans-retinoic acid (tRa) or hydrocortisone (H). Cells were lysed and subjected to immunoblot analysis with 4nM anti-hNIS antibody. A 100 kDa band of mature NIS and 60 kDa band of partially glycosylated polypeptide were detected.




NIS targeting to plasma membrane was assessed by immunofluorescence with anti-hNIS antibody. Cells were permeabilied with 0.2% BSA, 0.1% triton X-100 in PBS for 10min and then quenched with 50 mM NH4Cl in PBS for 10 min. Cells were incubated with 4nM anti-hNIS antibody for 1h at room temperature. Cells were washed and incubated with anti-rabbit FITC-conjugated secondary antibody. Nontreated MCF-7 (G); intracellular and faint plasma membrane-localized NIS expression in tRa-treated MCF-7 (H); clear plasma membrane localization of NIS in tRaH-treated MCF-7 (I).

**Quality Control by: LS**
**Quality Assurance by: JKM**
**Effective Date: 08-Dec-2016**

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Imanis Life Sciences
221 1st Ave SW STE 102
Rochester MN 55902
Tel: (507) 218-2559
Email: info@imanislife.com