ALPHA DIAGNOSTIC INTERNATIONAL

Product Specification Sheet

# Natural Resistance-Associated Macrophage Protein (Nramp2) Antibodies

| | | |
|---|---|---|
| Cat. # NRAMP24-P | Rat NRAMP2 Control/blocking Peptide # 4 | **SIZE**: 100 ug |
| Cat. # NRAMP24-S | Rabbit Anti-Rat NRAMP2 antiserum # 4 | **SIZE**: 100 ul |
| Cat. # NRAMP24-A | Rabbit Anti-Rat NRAMP2 IgG # 4 (affinity pure) | **SIZE**: 100 ug |

Natural resistance to infection with unrelated intracellular parasite such as Mycobacteria, Salmonella, and Leishmania is controlled by a single gene that encodes a macrophage-specific membrane protein designated as Natural Resistance-Associated Macrophage Protein (**Nramp1**). Recently a second member of NRAMP family, termed **NRAMP2/DMT/DCT1 (Divalent Metal ion Transporter 1 or Divalent Cation Transporter 1)**, has been identified (human, rat and mouse 568 aa, ~65% identity with NRAMP1). Unlike NRAMP1, NRAMP2 expression is more ubiquitous and has been detected in most tissues. It is dramatically up-regulated by iron starvation in the intestine. NRAMP2 gene produces two alternatively spliced transcripts generated by alternative use of two 3' exons encoding distinct C-termini of the protein as well as distinct 3' untranslated regions (UTRs). Interestingly, one Nramp2 mRNA contains an iron-responsive element (IRE) in its 3'UTR. The IRE is an RNA secondary structure present in the 5'- or the 3'-UTR of animal mRNAs encoding proteins involved in iron metabolism. The second Nramp2 splice isoform (**without-IRE, isoform II**) encodes a protein in which the C-terminal 18-aa of the IRE form (**with IRE**, **isoform I**) are replaced by a novel 25-aa segment and codes for a distinct 3' UTR lacking the IRE. The two isoforms are differentially localized and regulated in GI tract and kidney. It has recently been demonstrated that the Nramp2 gene is mutated (Gly185 to Arg at TM4) in both the mk and Belgarde (b) animal models exhibiting a severe microcytic hypochromic anemia marked by a defect in iron absorption by intestinal cells and in erythroid iron use.

## Source of Antigen and Antibodies

| | |
|---|---|
| **Antigen** | A 19-aa peptide of **rat NRAMP2** (protein accession #, refs 1) ; **Designated (NRAMP24 -P or control peptide)** conjugated to KLH; Epitope ~**Epitope location**~ Cytoplasmic domain #4 |
| **Ab Host/type** | Rabbit, Polyclonal antiserum # **NRAMP24** -S and IgG, purified over antigen-agarose (Cat # **NRAMP24**-A) |
| **2-Ab** | Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available). |
| **-ve control IgG** | Cat # 20009-1, Rabbit (non-immune) Serum IgG, purified, suitable for ELISA, Western, IHC as –ve control |

## Form & Storage of Antibodies/Peptide Control

**Antiserum (unpurified)**
100ul solution lyophilized powder
Supplied 0.05% azide, **Reconstitute** powder in 100 ul PBS

**Affinity pure IgG**
100 ug/100ul solution lyophilized powder
Supplied in **Buffer**: PBS+0.1% BSA
**Reconstitute powder** in PBS at 1mg/ml

**Control/blocking peptide**
100 ug/100 ul solution lyophilized powder
Supplied in Buffer: PBS pH 7.5,
**Reconstitute powder in PBS at 1 mg/ml.**

**Storage**

**Short-term**: unopened, undiluted liquid vials at -20OC and powder at 4oC or -20oC..

**Long-term**: at –20C or below in suitable aliquots after reconstitution. Do not freeze and thaw and store working, diluted solutions.

**Stability**: 6-12 months at –20oC or below.

## Recommended Usage

**Western Blotting** (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique).

**ELISA**: Control peptide can be used to coat ELISA plates at 1 ug/ml and detected with antibodies (1:10-50K for neat serum and 0.5-1 ug/ml for affinity pure).

**Histochemistry** & Immunofluorescence: Not tested. Affinity purified antibody at 5-20 ug/ml in paraformaldehyde fixed sections of tissues may be tested.

## Specificity & Cross-reactivity

The rat NRAMP24-P control peptide sequence is 100% conserved in mouse, 94% in human, 73% in zebra fish NRAMP2. This sequence is common in NRAMP2 with or without IRE isoforms. This antibody will not distinguish between these isoforms. Antibody cross-reactivity in various species has not been established. Control peptide, because of its low mol. Wt (<3 kDa), is not suitable for Western. It should be used for ELISA or antibody blocking experiments (use 5-10 ug control peptide per 1 ug of aff pure IgG or 1 ul antiserum) to confirm antibody specificity (see detailed protocol at the web site)..

**General References**: Gunshin H et al (1997) Nature 388, 482; Fleming MD et al (1998) PNAS 95, 1148; Kishi F et al (1997) Mol Immunol. 6, 224; Gruenshield S et al (1995) Genomics 25, 514; Fleming MD et al (1997) Nature Genet. 16, 383; Canonne-Hergaux F et al (1999) Blood 93, 4406 (review).

**Citations of for NRAMP2 (**see updated list at the web site)

Moos T, 2004, J. Neurochemistry 88, 1, 233-245, WB, IHC; Kovar J, 2006, Blood Cells, Molecules, and Diseases, 37, 95-99, WB, ; Moos T, 2006, J. Neurochem. 98, 1946-1958., WB, IHC; Moos T, 2004, J. Neurochemistry 88, 1, 233-245, WB, IHC; Roth JA, 2000, J. Neurosci. 20: 7595; Cheah JH, 2006, Neuron 51, 431-

*This product is for In vitro research use only.

**Related material available from ADI**

Antibodies NRAMP1/2, MTP1, Transferrin, and receptor, Ferritin, Defensins 1/2
NRAMP24-S-A-P 71223A

Alpha Diagnostic Intl Inc., 6203 Woodlake Center Dr, San Antonio, TX 78244, USA; **Email**: service@4adi.com
☎ (800) 786-5777; (210) 561-9515; **Fax**: (210) 561-9544; **Web Site**: www.4adi.com