ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

**Natural Resistance-Associated Macrophage Protein (Nramp2) Antibodies**

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| □ Cat. # NRAMP23-P | Rat NRAMP2 (without-IRE) Control Peptide # 3 | **SIZE**: 100 ug |
| □ Cat. # NRAMP23-S | Rabbit Anti-Rat NRAMP2 (without-IRE) antiserum # 3 | **SIZE**: 100 ul |
| □ Cat. # NRAMP23-A | Rabbit Anti-Rat NRAMP2 (without-IRE) IgG # 3 (aff 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

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| **Antigen** | A 22 AA **Peptide** (designated **NRAMP23-P**; **control peptide**) sequence located at the predicted **cytoplasmic** loop near the C-terminus of **rat NRAMP2, without IRE** (1) |
| **Ab Host/type** | Rabbit, Polyclonal antiserum # **NRAMP23**-S and IgG, purified over antigen-agarose (Cat # **NRAMP23**-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.
**Shipping**: 4oC for solutions and room temp for powder

## Recommended Usage

**Western Blotting** (1:1K-5K for neat serum and 1-10 ug/ml for affinity pure antibody using ECL technique). See refs in 2

**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: Affinity purified antibody at 5-20 ug/ml in paraformaldehyde fixed sections of tissues may be tested. See refs in 2

## Specificity & Cross-reactivity

The NRAMP23-P control peptide sequence is 81% conserved in human, mouse, and 77% in monkey NRAMP2 without IRE. This sequence is absent in NRAMP2 with IRE. No significant sequence homology exist with NRAMP1. 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**: (1). 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).

*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

Study **distribution of proteins in kidney** in 7 discrete region of rat kidney using pre-made protein blots.
NRAMP23-S-A-P 71223A

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