ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Hephaestin (Hp) Antibodies

½ **Cat.** # HEPH11-S	Rabbit Anti-Mouse Hp antiserum	**SIZE**: 100 ul
½ **Cat.** # HEPH11-A	Rabbit Anti-Mouse Hp IgG (aff pure)	**SIZE**: 100 ug
½ **Cat.** # HEPH11-P	Mouse Hp Control/blocking peptide	**SIZE**: 100 ug

Elemental iron is required for a variety of normal cellular functions and vital for proper growth and development. However, natural iron is quite insoluble and excess iron is harmful, since it can catalyze the formation of potentially damaging reactive oxygen species. Humans also have very limited capacity to excrete iron. Therefore, cells have developed mechanisms to improve solubility of iron and to control intracellular iron levels at the point of absorption in the intestine and other tissue. Several proteins including **Ferritin, transferrin (Tf), transferrin receptors (TfRs)**, and **iron regulatory proteins (IRPs**), iron transporter (**NRMAP2/DMT1/DCT1**) etc play a key role in iron metabolism. Some genes involved in iron-metabolism are associated with genetic disorders such as Friedreich's Ataxia (**Frataxin**), genetic hemochromatosis (**HFE**), and Sex-linked anemia (**Hephaestin**).

Hephaestin (Hp) gene was originally cloned as the gene defective in sex-linked anemia (sla) that is characterized by moderate to severe microcytic hypochronic anemia. Hp protein (mouse/rat 1157 aa, human 1158 aa, ~136 kDa) encodes a single-membrane spanning domain protein with extensive homology (50-60%) with copper containing serum ferrooxidase **ceruloplasmin (Cp).** TM domain is located very close to the C-terminus followed by the short cytoplasmic tail. Cp is involved in release of iron from various tissues. Hp is mostly expressed throughout the small intestine and colon. Low levels are also found in some other tissues (spleen, placenta and lung). It is localized in mature villus enterocytes with little or no expression in the crypts.

Source of Antigen and Antibodies

Antigen	18-aa peptide from mouse/**rat Hp (1)**; **Designation (#HEPH11-P, control/blocking peptide**) conjugated to KLH; epitope location ~ C-terminus
Ab Host/type	Rabbit, Polyclonal unpurified antiserum (#**HEPH11**-S) and IgG, purified over antigen-agarose (Cat # **HEPH11**-A)
2-Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
-ve control IgG	# 20009-1, Rabbit (non-immune) 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 antiserum and 1-10 ug/ml for aff. pure IgG using Chemiluminescence technique).

ELISA (1:100K; using 50-100 ng control peptide/well).

Histochemistry & Immunofluorescence: See refs using this antibody in refs. 2.

Specificity & Cross-reactivity

The mouse HEPH11-P sequence is 100% conserved in rat, bovine and 94% in human Hp. Antibody cross-reactivity in various species has not been studied. 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). Vulpe CD et al (1999) Nat. genet. 21, 195-199; Frazer DM et al (2001) Am. J. Physiol. 281, G931-G939;

2. Citations of for ADI Antibodies (see updated list at the web site)

Kovar J, 2006, Blood Cells, Molecules, and Diseases, 37, 95-99, WB,
Hahn P, 2004, Proc. Natl. Acad. Sci. 101: 13850 - 13855, WB, IHC
Reeves PG, 2005, Exp. Biol. Med., 230: 320 - 325, WB,
Hinoi T, 2005, Gastroenterology, 128, 946-961, WB,
Qian MZ, 2007, BBA 1772, 527-532, WB,
Reeves PG, 2005, J. Nutr., 135: 92 - 98, WB,

*This product is for in vitro research use only.

Some New Antibodies from ADI…

IRP1-2, HFE, Frataxin, Hepcidin, Hephaestin, NRAMPs, USF2, Ferritin, Light and heavy chains, ferritin and B2-M ELISA, Tfr1-2, ceruloplasmin, B2-Micro globulin,

HEPH11-S-A-P 150415A

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