ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

HFE Protein Antibodies

Cat. # HFE11-S	Rabbit Anti-Rat HFE antiserum	**SIZE**: 100 ul
Cat. # HFE11-A	Rabbit Anti-Rat HFE IgG (aff pure)	**SIZE**: 100 ug
Cat. # HFE11-P	Rat HFE 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**).

Hereditary hemochromatosis (HHC) is most common autosomal recessive disorder characterized by defective intestinal iron absorption, which lead to iron-overload in many tissues and toxic effects. The candidate gene for HHC encodes the **HFE protein** (formerly called **HLA-H**) resembling the major histocompatibility complex MHC class-1 molecule. HFE protein (mouse 359 aa, rat 360 aa, human 348 aa, ~48 kDa) is type I membrane protein. It is found in all tissues except brain. HFE protein binds too Tfr and reduces its affinity for iron-loaded Tf. The HFE Cys282-Tyr (C282Y) is homozygous in 83-100% of HHC subjects in the US and North Europe, and Australia. The C282Y mutation results in the loss of a structural disulfide bond in the alpha-3 domain of the protein, which prevents association with **beta-2 microglobulin** and proper presentation to the surface. Defects in HFE are also a cause of porphyria cutanes tarda (PCT), a disorder characterized by light-sensitive dermatitis and presence of large amounts of uroporphyrin in urine.

Source of Antigen and Antibodies

Antigen	An 18-aa peptide sequence (**designated HFE11-P or control peptide**) within the C-terminus of **rat HFE** (1) was synthesized, coupled to KLH
Ab Host/type	Rabbit, Polyclonal antiserum # **HFE11**-S and IgG, purified over antigen-agarose (Cat # **HFE11**-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 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: Not tested

Specificity & Cross-reactivity

The rat HFE11-P sequence is 100% conserved in rat HFE protein homolog RT1-CAFÉ (360-aa), HFE splice variants delE2 (14E4) (172 aa), 83% in mouse, 72% in rhinoceros, and 61% in human HFE. We recommend the use of cat # HFE12-A for human HFE. 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). Riegert P et al (1998) Immunogenetics 47, 174; Hashimoto K et al (1997) BBRC 230, 35; Feder Jn et al (1996) Nat. genet. 13, 399; Roberts AG et al (1997) Lancet 349, 321; Zuccpn L et al (2000) Haematologia 85, 346; Parkkila S et al (2000) Haematologia 85, 340; Griffiths W (2000) Hum. Mol. Genet. 9, 2377

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

Martin PM, 2006, Invest. Ophthalmol. Vis. Sci., Oct 2006; 47: 4238 – 4244, WB, IF, mouse retina and RPE, co localization and double labeling

*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,
HFE11-S-A-P 71214A

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