ALPHA DIAGNOSTIC INTERNATIONAL

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Product Specification Sheet

Heme Oxygenase-1 (HO-1) Antibodies

Cat. # HO11-S	Rabbit Anti-Rat HO-1 antiserum # 1	**SIZE**: 100 ul
Cat. # HO11-A	Rabbit Anti-Rat HO-1 IgG # 1(aff pure)	**SIZE**: 100 ug
Cat. # HO11-P	Rat HO-1 Control/blocking peptide # 1	**SIZE**: 100 ug
Cat. # HO11-C	Recombinant purified Rat HO-1 protein WB +ve control	**SIZE**: 100 ul

Heme oxygenase is the rate-limiting microsomal enzyme in the heme degradative pathway. Heme oxygenase catalyzes the NADPH, O2 and cytochrome P450 reductase dependent oxidation of heme to form equimolar biliverdin, carbon monoxide, a putative neurotransmitter, and iron. Biliverdin is subsequently converted to bilirubin by biliverdin reductase. These products of the HO reaction have important physiological effects: carbon monoxide is a potent vasodilator; biliverdin and its product bilirubin are potent antioxidants; "free' iron increases oxidative stress and regulates the expression of many mRNAs (e.g., DCT-1, ferritin and transferrin receptor) by affecting the conformation of iron regulatory protein (IRP)-1 and its binding to iron regulatory elements (IREs) in the 5'- or 3'-UTRs of the mRNAs. To date, 3 forms of **heme oxygenases (HO1-3)** have been identified. **HO-1 or Hsp-32** (EC 1.14.99.3; mouse/rat 289 aa; human 288 aa, chromosome 22; ~88% homology between the species) is an inducible enzyme. Ho-1 is expressed in most tissues with highest levels in spleen. HO-1 gene expression is inducible by heme, suggesting an important role of HO-1 in heme metabolism. Many other agents or conditions related to oxidant damage such as longer wavelength UV radiation, hyperoxia, hypoxia, hydrogen peroxide, glutathione depletion, endotoxin, and, more recently, nitric oxide (NO) have also been found to stimulate HO-1 expression. HO-1 expression has been shown to increase in benign prostatic hyperplasia (BPH) and malignant prostate tissue.

Source of Antigen and Antibodies

Antigen	13-aa peptide from **rat HO1 (1); Designation (HO11-P, control peptide)** coupled to KLH; epitope location ~ N-terminus
Antibody host/type	Rabbit, Polyclonal unpurified antiserum (Cat # HO11-S); Rabbit, Polyclonal IgG (Cat # HO11-A), purified over antigen-Agarose
Secondary Ab	Cat # 20320, goat anti-rabbit IgG-HRP (AP, biotin, FITC conjugates also available).
Negative Control Ab	Non-immune rabbit IgG (Cat # 20009-1) to be used as –ve control for ELISA, WB, IHC etc.

Rat HO-1 (1-261 aa without TM domain) was expressed in E. colli and purified >95%. For **western blot +ve control (Cat # HO11-C)**, it is supplied in SDS-PAGE sample buffer (reduced). Load ~10 ul/lane to visualize with appropriate antibodies. This preparation is not biologically inactive. Store at –20oC in suitable aliquots. Avoid repeated thawing or heating.

Form & Storage of Antibodies/Peptide Control

Antiserum (unpurified)
100ul solution lyophilized powder
Supplied in Buffer: 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 using Chemiluminescence technique.

ELISA (1:10K-1:100K; using 50-100 ng of control peptide/well).

Histochemistry & Immunofluorescence. We recommend the use of affinity purified antibody at 10-30 ug/ml in formaldehyde fixed, paraffin-embedded tissues (1).

Specificity & Cross-reactivity

The rat HO11-P sequence is 92% conserved in human and mouse HO-1. HO11-P has no significant homology with HO-2. Antibody cross-reactivity in various species has not been studied. Control peptides, because of its small size (2-3 kDa), is not recommended for Western. It should be used in ELISA or antibody blocking (use 5-10 ug control peptide per 1 ug of IgG or 1 ul of antiserum) experiments to demonstrate antibody specificity (see detailed protocol at the web site).

General References: (1). Muller Rm et al (1987) JBC 262, 6795-6802; Shibahara S et al (1985) PNAS 82, 7865-7869; Kageyama H et al (1988) Cancer Res. 48, 4795-4798; Alam J et al (1994) JBC 269, 1001-1009; Keyse SM et al (1989) PNAS 86, 99-103; Yoshida T et al (1988) Eur. J. Biochem. 171, 457-461; 171, 457

*This product is for in vitro research use only.

HO11-S-A-P-C 71221J

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Opposite M2K Pitampura, Delhi – 110034 (INDIA). Ph: +91-11-42208000, 42208111, 42208222, Mobile: +91-9810521400, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com Website: www.lifetechindia.com