Introduction and Background

A. Overview

Heme Oxygenase-1 (HO-1), also known as Hsp32, is the inducible isoform of heme oxygenase that catalyzes the NADPH, O2 and cytochrome P450 reductase dependent oxidation of heme to carbon monoxide, ferrous iron and biliverdin which is rapidly reduced to bilirubin. These products of the HO reaction have important physiological effects: carbon monoxide is a potent vasodilator and has been implicated to be a physiological regulator of cGMP and vascular tone; 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 transferring 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, three identified heme oxygenase isoforms are part of the HO system that catalyze heme into biliverdin and carbon monoxide. These are inducible HO-1 or Hsp32, constitutive HO-2 that is abundant in the brain and testis, and HO-3 which is related to HO-2 but is the product of a different gene. The HO system is the rate-limiting step in heme degradation and HO activity decreases the levels of heme which is a well known potent catalyst of lipid peroxidation and oxygen radical formation[1,2,3]. The expression of HO-1 is highly responsive to all types of stimuli that cause oxidative stress and it is up regulated during exposure to oxidants, UV-A irradiation and a series of agents including cytokines, hormones, heme and heavy metals[1,4.] HO-1 is a vital component of neuronal defense mechanisms and oxidative stress has been postulated to be the underlying basis for neuronal cell death in neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease5. The expression of HO-1 is normally very low in the brain but increases markedly after heat shock, ischemia or glutathione depletion[2,6,7]. Spatial distribution of HO-1 expression in AD brain is essentially identical to that of the pathogenic conformational changes of tau protein that is the major component of the intraneuronal lesion of AD, neurofibrillary tangles8. HO-1 expression and tau expression may be regulated by oxidative stresses in a coordinated manner and play a pivitol role in the cytoprotection of neuronal cells9. Plasma and cerebrospinal fluid HO-1 protein and lymphocyte HO-1 mRNA levels are decreased in subjects with sporadic AD relative to normal elderly controls suggesting that measurement of HO-1 may serve as a useful biological marker in early sporadic AD[10].

Oxidative stress in the heart caused by ischemia and reperfusion has been shown to lead to cardiomyocyte death. An absence of HO-1 has detrimental consequences whereas overexpression of HO-1 plays a protective role in hypoperfusion and ischemia/reperfusioninduced myocardial injury[11,12]. Under normal conditions, HO-1 is present at low levels in all organs except the spleen, but its expression is rapidly accelerated in response to pathophysiological conditions such as renal ischemia/reperfusion and cellular transformation13. HO-1 overexpression exerts beneficial cytoprotective effects in a number of transplantation models, including antigen-independent ischemia/reperfusion injury, acute and chronic allograft rejection and xenotransplantation[14,15]. The mechanisms by which HO-1 confers its protective effects are currently poorly understood but this area of investigation is active and rapidly evolving. The measurement of HO-1 in various cell types, tissues and bodily fluids may provide new insights into the physiological roles of HO-1 and may

lead to monitoring HO-1 levels as a biomarker for therapeutic interventions or as an environmental biomarker in toxicology studies.

B. Test Principle

The HO-1 (rat), EIA kit is a colorimetric immunometric enzyme immunoassay kit with results in < 3 hours. Absorbance is read at 450 nm. Save time, money, and precious samplewith fully quantitative results and increased sensitivity compared to Western blot analysis.

C. Procedural Guidelines

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

This kit is not recommended for measuring human or mouse HO-1. Human HO-1 should be measured using the Human HO-1 ELISA kit

Material and Method

A. List of component

- Anti-Rat HO-1 Immunoassay Plate,
- 5X HO-1 Extraction Reagent
- Recombinant Rat HO-1 Standard
- Sample Diluent
- 10X Wash Buffer
- Anti-HO-1
- Anti-HO-1 Diluent
- Anti-Rabbit IgG:HRP Conjugate
- Anti-Rabbit IgG:HRP Conjugate Diluent
- TMB Substrate
- Acid Stop Solution

B. Storage

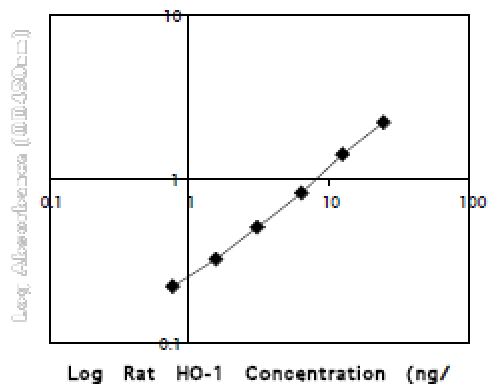
All reagents are stable as supplied at 4℃, except the Recombinant Rat HO-1 Standard, which should be stored at -20℃. For optimum storage, the Recombinant Rat HO-1 Standard should be aliquotted into smaller portions and stored at -20℃.

Protocol

1. Add Rat HO-1 Standards and samples in duplicate to the pre-coated ready-to-use Anti-Rat HO-1 Immunoassay Plate; incubate then wash.
2. Add diluted Anti-HO-1; incubate then wash.
3. Add diluted Anti-Rabbit IgG: HRP Conjugate; incubate then wash.

4. Develop with TMB Substrate.
5. Add Acid Stop Solution.
6. Measure absorbance at 450nm.
7. Plot HO-1 standard curve and determine sample concentrations.
8.

<u>Typical Standard Curve</u>



Performance Characteristics

<u>Number of Samples</u>: 1 standard curve, 40 samples in duplicate or 2 standard curves and 32 samples in duplicate

<u>Sensitivity</u>: 0.78ng/mL Range: 0.78-25ng/mL

<u>Sample Volume</u>: 100μL/well Incubation Time: <3 hours

<u>Wavelength Setting</u>: 450nm

<u>Specificity</u>: Recognizes rat HO-1; no reactivity with human or mouse HO-1.

<u>Species Reactivity</u>: Rat

<u>Sample Types</u>: Cell lysates, tissue extracts