

# HO-1 ELISA Kit



## TECHNICAL SPECIFICATIONS

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### Human HO-1 ELISA Kit

Product #: EKS-800

A sandwich immunoassay for the detection and quantitation of Human HO-1 protein in cell lysates and tissue extracts.

#### Specificity

Recognizes human HO-1; no reactivity with human HO-2.

#### Species Reactivity

Human

#### Sample Types

Cell lysates, tissue extracts

#### Scientific Background

Heme Oxygenase-1 (HO-1), also known as Hsp32, is the inducible isoform of heme oxygenase that catalyzes the NADPH, O<sub>2</sub> 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 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, 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<sup>1,2,3</sup>. 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<sup>4</sup>. 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 diseases<sup>5</sup>. The expression of HO-1 is normally very low in the brain but increases markedly after heat shock, ischemia or glutathione depletion<sup>2,6,7</sup>. 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 tangles<sup>8</sup>. HO-1 expression and tau expression may be regulated by oxidative stresses in a coordinated manner and play a pivotal role in the cytoprotection of neuronal cells<sup>9</sup>. 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<sup>10</sup>.

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/reperfusion-induced myocardial injury<sup>11,12</sup>. 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 transformation<sup>13</sup>. 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<sup>14,15</sup>.

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.

#### Background References

References are available upon request.

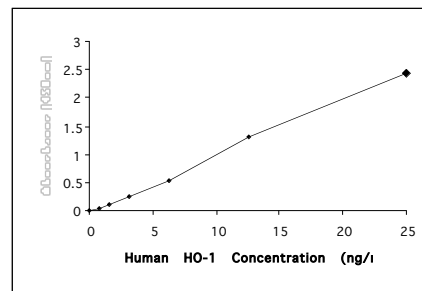
#### Application Note

This kit is not recommended for measuring mouse or rat HO-1.

#### Assay Procedure Summary

1. Add HO-1 Standards and samples in duplicate to the pre-coated ready-to-use Anti-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 Stop Solution.
6. Measure absorbance at 450nm.
7. Plot HO-1 standard curve and determine sample concentrations.

#### Typical Standard Curve



#### References Citing EKS-800

1. Kravets, A.; Hu, Z.; Miralem, T.; Tomo, M.D.; Maines, M.D.; (2004) *J. Biol. Chem.* **279**: 19916-19923.
2. Philippidis, P.; Mason, J.C.; Evans, B.J.; Nadra, I.; Taylor, K.M.; Haskard, D.O.; Landis, R.C.; (2004) *Circ. Res.* **94**: 119-126.

Rev: 03/30/06

## CERTIFICATE OF ANALYSIS

### HO-1 ELISA Kit

Product #: EKS-800

Size: 1 X 96 well plate

Lot#:

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

Sensitivity: 0.78ng/mL

Range: 0.78-25ng/mL

Sample Volume: 100µL/well

Incubation Time: <2.5 hours

Wavelength Setting: 450nm

Materials Included:

- Anti-HO-1 Immunoassay Plate, Lot# 03150654
- 5X Extraction Reagent, Lot# 03060605
- Human HO-1 Standard, Lot# 03220606
- Sample Diluent, Lot# 03070602
- 20X Wash Buffer, Lot# 01180605
- Anti-Human HO-1, Lot# 03140603
- Antibody Diluent, Lot# 03060607
- HRP Conjugate, Lot# 03150646
- HRP Conjugate Diluent, Lot# 03030624
- TMB Substrate, Lot# 12190501
- Stop Solution 2, Lot# 12140501
- Instruction Manual

Storage of Kit: All reagents are stable as supplied at 4°C, except the Recombinant HO-1 Standard, which should be stored at -20°C. For optimum storage, the Recombinant HO-1 Standard should be aliquotted into smaller portions and stored at -20°C.



**FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES:** This product is not to be used for diagnostic nor therapeutic purposes. By accepting this product, the user confirms that it will be used for research purposes only. Stressgen warrants that its products conform to the information published by Stressgen. Purchaser must determine the suitability of the product for their particular use. Please refer to the MSDS for product safety information.

# MATERIAL SAFETY DATA SHEET

## Section 1 - Product Identification and Use

**Product Name: Human HO-1 ELISA Kit**

**Product#: EKS-800**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: Stressgen Bioreagents Limited Partnership  
Street Address: 120-4243 Glanford Avenue  
City, Prov. Postal Code: Victoria, B.C., CANADA V8Z 4B9  
Fax: (250) 744-2877  
EMERGENCY PHONE: (250) 744-2811 Toll Free in North America: 1-800-661-4978

## Section 2 - Hazardous Ingredients

Hazardous Ingredients:

Kit components may contain the following components:

CAS Registry Numbers	Chemical Name	Percentage
26628-22-8	Sodium Azide	0.1
26172-55-4	5-chloro-2-methyl-isothiazolin-3-one	<<1.15
2682-20-4	2-methyl-4isothiazolin-3-one	<<0.35
10031-43-4	Copper (II) nitrate	<<0.35
7786-30-3	Magnesium Chloride	<<1.0
1.077-60-3	Magnesium Nitrate	<<1.7
9016-45-9	Nonylphenylpolyethylene glycol	<12.5
54827-17-7	3,3,5,5-tetramethylbenzidine (TMB)	N/A
7647-01-0	Hydrogen chloride	<8.5

## Section 3 - Physical Data

The physical properties of the kit components have not been investigated thoroughly.

TMB: Clear liquid

Stop Solution: Clear, colorless to slight yellow, liquid

## Section 4 - Fire and Explosion Hazard

Components of the kit are non-flammable. Fire and explosion data have not been investigated.

Hazardous Combustion Products:

Kit components may emit toxic vapors

TMB: carbon monoxide, carbon dioxide, nitrogen oxide fumes

Stop Solution: Carbon dioxide, carbon monoxide, hydrogen gas fumes

## Section 5 - Reactivity Data

Kit components are stable. Avoid contact with reducing agents, amines, strong acids, strong bases, and strong oxidizing agents.

TMB: Stable. Protect from direct UV light, avoid elevated temperatures, protect from moisture. Incompatible with strong oxidizing agents, metals.

Stop Solution: Stable. Avoid elevated temperatures. Incompatible with oxidizing agents, reducing agents, and bases.

# MATERIAL SAFETY DATA SHEET

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## Section 6 - Toxicological Properties

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The toxicological properties of the kit components have not been investigated. Kit components may burn eyes severely, be harmful if absorbed through skin, burn skin severely, irritate respiratory tract, be harmful if inhaled, be harmful if swallowed. May cause an allergic reaction in some individuals.

**TMB:** Irritant to eyes and skin. May cause irritation of the digestive tract and respiratory tract.

**Stop Solution:** Corrosive to skin and eyes. Inhalation of vapors may cause irritation of nasal and respiratory tract. Ingestion causes gastrointestinal tract discomfort, nausea and vomiting. IARC: Group 3 Carcinogen

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## Section 7 – Spill and Disposal

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Wear chemical safety glasses or goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.

\*\*\*\*\*MULTIPLE COMPONENT SPILL OR LEAK PROCEDURES\*\*\*\*\*

- Wear protective equipment (respirator, chemical safety goggles, rubber boots, and heavy rubber gloves).
  - Ventilate area.
  - Absorb on sand or vermiculite and place in closed containers for disposal.
  - Dispose or mix the material with a combustible solvent and burn in a chemical incinerator equipped with an afterburner and scrubber.
  - Containers of this material may be hazardous when emptied. Emptied containers retain product residues: handle as if they were full.
  - Observe all federal, state and local environmental regulations.
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## Section 8 - First Aid Measures

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- If any of the kit components are ingested, immediately rinse mouth with water and seek medical attention.
  - In case of skin contact, flush with copious amounts of water for at least 15 minutes. Remove contaminated clothing and shoes. If a rash or other irritation develops, seek medical attention.
  - If inhaled, remove to fresh air. If breathing becomes difficult, seek medical attention.
  - In case of eye contact, flush with copious amounts of water for at least 15 minutes while separating the eyelids with fingers. Seek medical attention.
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## Section 9 - Preparation

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Prepared by: Stressgen Bioreagents Limited Partnership	Phone#: (250) 744-2811 Toll free in North America: 1-800-661-4978	Created: 06/03/05	Modified: 03/30/06
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The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. Stressgen shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalogue for additional terms and conditions of sale.