

**Revised 8 May 2009 (Vers. 1.0)**

This ImmunoSet contains the basic components for the development of a HO-1 (human) immunometric enzyme immunoassay (EIA) .

Each kit contains sufficient reagents for five 96-well plates.

This kit has been validated for use with cell lysates and microsomes. Additional sample types will require validation by the user.

## **1 INTRODUCTION**

Heme oxygenase (Hsp32 ) is the rate-limiting enzyme that breaks down heme to iron, carbon monoxide, and biliverdin, which is then metabolized to bilirubin by biliverdin reductase<sup>1,2</sup>. In mammals, heme oxygenase exists as two primary isoforms, the inducible isoform HO-1, and the constitutively expressed HO-2, both catalyzing the same reaction.

HO-1 is expressed in erythrocyte and haemoglobin metabolizing tissues of the spleen, liver, and bone marrow, with localization to membranes of the ER, mitochondria, and caveolae<sup>2</sup>. HO-1 expression is induced in response to an array of oxidative stress-inducing factors, including heat shock, heme accumulation, hypoxia, UV radiation, nitric oxide, cytokines, and heavy metals.

## **2 REFERENCES**

1. Abraham, N.G. and Kappas, A. (2008). *Pharmacol Rev.* 60, 79-127.
2. Ryter, S.W., et al. (2006) *Physiol Rev.* 86. 583-650.

## **3 MATERIALS PROVIDED**

1. **HO-1 (human) Capture Antibody**  
One vial containing 219 µg lyophilized HO-1 (human) monoclonal antibody
2. **HO-1 (human) Standard**  
One vial containing 156.25 ng of recombinant HO-1 (human) protein in 0.25 mL buffer ,
3. **HO-1 (human) Detection Antibody**  
One vial containing 18.75 µg lyophilized HO-1 (human) polyclonal antibody
4. **SA-HRP**  
One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase

## **4 MATERIALS NEEDED BUT NOT SUPPLIED**

1. RIPA Cell Lysis Buffer, or similar
2. 96-well high-binding polystyrene microtiter plates, Cat. #80-1930, or similar
3. Precision pipets
4. Microplate reader capable of reading at 450 nm
5. Phosphate buffered saline (PBS)<sup>†</sup>
6. Tween®-20<sup>\*†</sup>
7. Bovine Serum Albumin (BSA)<sup>†</sup>
8. 3,3',5,5' tetramethylbenzidine (TMB) solution, Cat. #80-1805 or similar<sup>†</sup>
9. 1N hydrochloric acid, such as Stop Solution, Cat. #80-1804<sup>†</sup>

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† ImmunoSet Buffer Pack, Cat. #950 -003

\* Tween is a registered trademark of ICL Americas

**5 BUFFER FORMULATIONS****1. Coating Buffer**

10 mM sodium phosphate, 15 mM NaCl, pH 7.4

**2. Blocking Buffer**

10 mM sodium phosphate, 15 mM NaCl, 1.0% BSA, pH 7.4

**3. Assay Buffer**

100 mM sodium phosphate, 150 mM NaCl, 1.0% BSA, 0.1% Tween-20, pH 7.4

**4. Wash Buffer**

10 mM sodium phosphate, 15 mM NaCl, 0.1% Tween-20, pH 7.4

**6 PLATE COATING**

1. Reconstitute HO-1 (human) Capture Antibody with 250  $\mu$ L deionized water for a 250x stock. Use immediately, or make aliquots and freeze at  $-20^{\circ}\text{C}$  for up to 3 months. For prolonged storage, aliquot and freeze at  $-80^{\circ}\text{C}$ . Avoid repeated freeze/thaw cycles.
2. Dilute the stock 1:250 in Coating Buffer. Immediately dispense into 96-well microtiter plates using 100  $\mu$ L of the diluted capture antibody per well. Seal the plate and incubate overnight at room temperature.
3. Aspirate each well to remove coating solution. Immediately add 200  $\mu$ L Blocking Buffer per well. Seal the plate and incubate for at least 1 hour.
4. Aspirate each well to remove blocking solution. Plates may be used immediately or dried and stored with desiccant at  $4^{\circ}\text{C}$ .

**7 REAGENT PREPARATION****1. Recombinant HO-1 (human) Standard**Standard is supplied as a 50x stock. Aliquot and store at  $-20^{\circ}\text{C}$ .

Refer to kit expiration date for standard expiry.

For prolonged storage, aliquot and freeze at  $-80^{\circ}\text{C}$ . Avoid repeated freeze/thaw cycles.

The recommended standard curve range is 12.5 ng/mL to 0.195 ng/mL, using 2-fold serial dilutions in Assay Buffer.

Do not store diluted standard.

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**2. HO-1 (human) Detection Antibody**Reconstitute vial contents with 250  $\mu$ L deionized water for a 250x stock.

Store at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

Dilute the stock 1:250 in Assay Buffer for a working solution. Do not store diluted antibody.**3. SA-HRP**Reconstitute vial contents with 250  $\mu$ L deionized water for a 600x stock.

Store at 4°C for up to 3 months. For prolonged storage, aliquot and freeze at -20°C. Avoid repeated freeze/thaw cycles.

Dilute the stock 1:600 in Assay Buffer for a working solution. Do not store diluted conjugate.**8 ASSAY PROCEDURE**

1. Pipet 100  $\mu$ L of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100  $\mu$ L of standards and samples, prepared in Assay Buffer, to the bottom of the appropriate wells.
3. Seal the plate. Incubate for 1 hour at room temperature.
4. Empty the contents of the wells and wash by adding 400  $\mu$ L of Wash Buffer to every well.  
Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
5. Pipet 100  $\mu$ L of the diluted detection antibody into each well, except the blank.
6. Seal the plate. Incubate for 1 hour at room temperature.
7. Wash as above (Step 4).
8. Add 100  $\mu$ L of the diluted conjugate to each well except the blank.
9. Seal the plate. Incubate for 30 minutes at room temperature.
10. Wash as above (Step 4).
11. Pipet 100  $\mu$ L of TMB solution into each well.
12. Seal the plate. Incubate for 30 minutes at room temperature.
13. Pipet 100  $\mu$ L 1N HCl into each well.
14. After blanking the plate reader against the substrate, read optical density at 450 nm. If the plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.



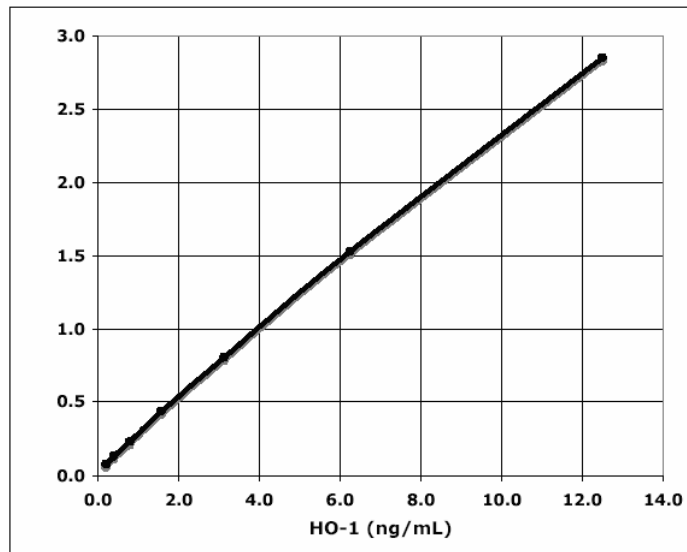
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## 9 ASSAY PERFORMANCE

### 9.1 Typical Data

The results shown below are for illustration only and should not be used to interpret results from another assay.



### 9.2 Sensitivity

The sensitivity, or limit of detection, of this assay is 0.049 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 7 standard curves.

### 9.3 Specificity

This assay detects HO-1 in cell lysates and microsomes of human origin.

There is no cross reactivity with human HO-2 or HO-3.

### 9.4 Dilutional Linearity

To determine possible interference from the sample matrix, the indicated sample types were serially diluted into assay buffer. The concentrations of human HO-1 were measured in the assay, and the results were analyzed to determine the range over which a linear response was obtained. These data may be used as a guideline to determine minimal recommended dilution (MRD) for similar samples.

Dilution Factor	Hela CL	Liver MS
Neat	104%*	29%**
1:2	97%	57%
1:4	109%	74%
1:8	100%	78%
1:16		88%
1:32		88%
1:64		96%
1:128		100%

CL: Cell Lysate MS: Microsomes

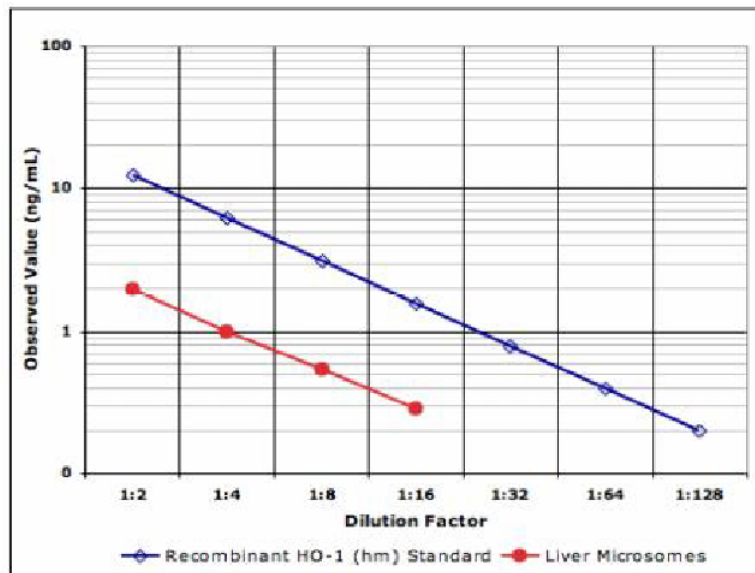
\*Cell lysate was diluted 1: 80 in Assay Buffer for levels to be within the dynamic range of the assay.

\*\*Microsomes were diluted 1:10 in Assay Buffer for levels to be within the dynamic range of the assay.

**9.5 Parallelism**

Dose-response curves from microsomes diluted into assay buffer (using the MRD) were compared to the recombinant human HO-1 standard curve.

Parallelism indicates antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of analyte.



**9.6 Calculation of Results**

Several options are available for the calculation of the relative levels of HO-1 in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve-fitting program. For accuracy, please ensure that sample values fall within the standard range.



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**10 ACCESSORY REAGENT LIST**

Reagent	Quantity	Cat. #
Immuno Set Buffer Pack	1 each of the following products: 80-1927, 80-1928, 80-1929, 80-1805, 80-1804	950-003
Immuno Set Plate Pack	5 x 96-well clear microtiter plates & 5 plate sealers	80-1930
PBS Concentrate	120 mL	80-1927
BSA Solution (10%)	50 mL	80-1928
Tween-20 Solution (10%)	30 mL	80-1929
RIPA Cell Lysis Buffer 2	100 mL	80-1284
Wash Buffer Concentrate	100 mL	80-1287
SA-HRP	12.5 µg/vial	80-1896

**11 STORAGE**

Store all components at 4°C, except for the standard.

Standard must be stored frozen at -20°C.

See section 7 “REAGENT PREPARATION” for storage of reconstituted material.

**12 TIPS & TROUBLESHOOTING**

- If buffers other than those recommended are used in the assay, the end-user must determine the appropriate dilution and assay validation.
- Pipet the reagents to the sides of the wells to avoid possible contamination.
- Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.
- Insufficient washing or residual wash buffer in the wells may cause variation in assay results.
- Bring all reagents to room temperature for at least 30 minutes prior to opening.
- All standards, controls, and samples should be assayed in duplicate.

**13 LIMITED WARRANTY**

DRG warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.



**DRG<sup>®</sup> HO-1 (human) Basic (EIA-5064)**













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









DRG must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if DRG is not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy to the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

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### SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
	European Conformity	CE-Konformitätskennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
				
	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilstrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstemperatur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Distributed by				
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθό..