> ImmunoSet® HO-1 (mouse), ELISA development set

Catalog # ADI-960-071
Reagents for 5 x 96-Well EIA Kits

This ImmunoSet contains the basic components for the development of a HO-1 (mouse) immunometric enzyme immunoassay (EIA).* Each kit contains sufficient reagents for five 96-well plates.

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

Visit www.enzolifesciences.com for tips and frequently asked questions.

*See Specificity under Assay Performance, page 5, for sequence homology of mouse and rat HO-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[1,2]. In mammalians, 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 hemoglobin metabolizing tissues of the spleen, liver, and bone marrow, with localization to membranes of the ER, mitochondria, and caveolae[2]. 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.

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.

Materials Provided

1. HO-1 (mouse) Capture Antibody

   One vial containing 187.5 µg lyophilized HO-1 (mouse) polyclonal antibody, Cat. #80-1937

2. HO-1 (rat) Standard

   One vial containing 156.25 ng lyophilized recombinant HO-1 (rat) protein, Cat. #80-1911

3. HO-1 (mouse) Detection Antibody

   One vial containing 18.75 µg lyophilized HO-1 (mouse) polyclonal antibody, Cat. #80-1938

4. SA-HRP

   One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase, Cat. #80-1896

Materials Needed but not Supplied

1. RIPA Cell Lysis Buffer, Cat. #80-1284, or similar
2. Igepal CA-630, Sigma Cat. #I3021, or similar
3. 96-well high-binding polystyrene microtiter plates, Cat. #80-1930, or similar
4. Precision pipets
5. Microplate reader capable of reading at 450 nm
6. Phosphate buffered saline (PBS)†
7. Tween®-20*†
8. Bovine Serum Albumin (BSA)†
9. 3,3',5,5' tetramethylbenzidine (TMB) solution, Cat. #80-1805 or similar†
10. 1N hydrochloric acid, such as Stop Solution 2, Cat. #80-1804†

†ImmunoSet Buffer Pack, Cat. #ADI-950-003

*Tween is a registered trademark of ICL Americas

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

Plate Coating

1. Reconstitute HO-1 (mouse) Capture Antibody with 250 µL deionized water for a 250x stock. Use immediately, or make aliquots and freeze at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°C. Avoid repeated freeze/thaw cycles.
2. Dilute the stock 1:250 in Coating Buffer. Immediately dispense into 96-well microtiter plates using 100 µL 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 µL 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°C. A moderate increase in signal may be seen if wet plates are used.

Reagent Preparation

1. Recombinant HO-1 (rat) Standard

   Reconstitute vial contents with 250 µL deionized water for a 625 ng/mL (50x) stock. Aliquot and store at -20°C for up to 3 months. For prolonged storage, aliquot and freeze at -80°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.

2. HO-1 (mouse) Detection Antibody

   Reconstitute vial contents with 250 µL 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. For optimal results, prepare the working solution at the time of standard

curve preparation. Do not store diluted antibody past the day of dilution.

3. SA-HRP

   Reconstitute vial contents with 250 µL 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.

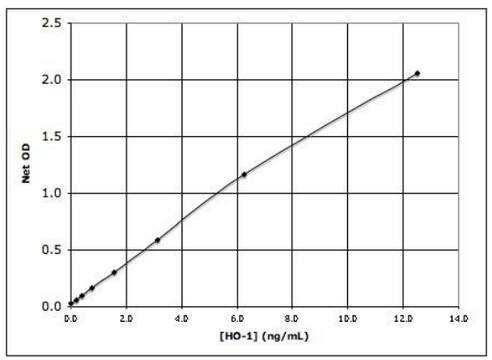
Assay Procedure

1. Pipet 100 µL of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100 µL 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 µL 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 µL 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 µL 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 µL of TMB solution into each well.
12. Seal the plate. Incubate for 30 minutes at room temperature.
13. Pipet 100 µL 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

Assay Performance

Typical Data

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



Sensitivity

The sensitivity, or limit of detection, of this assay is 0.096 ng/mL. It was determined by interpolation at 2 standard deviations above the mean signal at background, using data from 8 standard curves.

Specificity

This assay detects natural HO-1 in cell lysates, serum, EDTA plasma, and microsomes of mouse origin. The standard is recombinant rat HO-1 (accession number P06762), which has 93% sequence identity and 95% sequence homology to mouse HO-1 (accession number P14901).

Cross reactivity with rat HO-2 is less than 0.02%. Rat HO-2 (accession number P23711) has 96% sequence identity and 98% sequence homology to mouse HO-2 (accession number O70252).

Dilutional Linearity

To determine possible interference from the sample matrix, the indicated sample types were serially diluted into the assay buffer. The concentrations of mouse 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	3T3 CL	S†	EDTA P†	Liver MS
Neat	---	67%	71%	97%*
1:2	69%	84%	80%	96%
1:4	86%	87%	94%	97%
1:8	102%	100%	100%	100%
1:16	100%	---	---	---

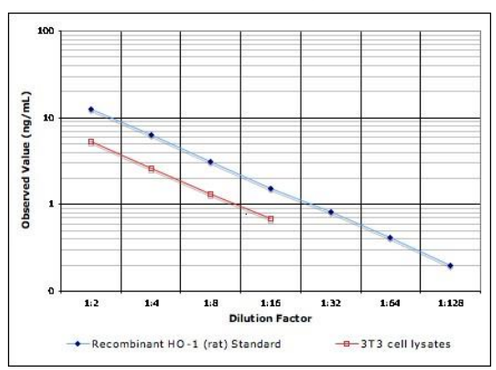
CL: Cell Lysate, **S:** Serum, **P:** Plasma, **MS:** Microsomes

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

†Serum and EDTA plasma samples were treated with 0.5% Igepal CA-630 prior to assaying.

Parallelism

Dose-response curves from cell lysates diluted into assay buffer (using the MRD) were compared to the recombinant rat HO-1 standard curve. Parallelism indicates antibody-binding characteristics of the native and standard proteins are similar, allowing accurate determination of analyte.



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.

Accessory Reagent List		
Reagent	Quantity	Cat. #
ImmunoSet® Buffer Pack	1 each of the following products: 80-1927, 80-1928, 80-1929, 80-1805, 80-1804	ADI-950-003
ImmunoSet® Plate Pack	5 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

Storage

Store all components at 4°C. See page 3 for storage of reconstituted material.

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.

Limited Warranty

Enzo Life Sciences International, Inc. makes no warranty of any kind, expressed or implied, which extends beyond the description of the product in this brochure, except that the material will meet our specifications at the time of delivery. Enzo Life Sciences International, Inc. makes no guarantee of results and assumes no liability for injuries, damages or penalties resulting from product use, since the conditions of handling and use are beyond our control.

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