This ImmunoSet contains the basic components for the development of a HO-1 (rat) 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.

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 (rat) Capture Antibody

   One vial containing 281 µg lyophilized HO-1 (rat) monoclonal antibody.

2. HO-1 (rat) Standard

   One vial containing 156.25 ng lyophilized recombinant HO-1 (rat) protein.

3. HO-1 (rat) Detection Antibody

   One vial containing 4.7µg lyophilized HO-1 (rat) polyclonal antibody.

4. SA-HRP

   One vial containing 12.5 µg lyophilized streptavidin conjugated to horseradish peroxidase.

Materials Needed but not Supplied

1. RIPA Cell Lysis Buffer or similar.
2. Igepal CA-630 or similar.
3. 96-well high-binding polystyrene microtiter plates 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 or similar†
10. 1N hydrochloric acid, such as Stop Solution 2†

†ImmunoSet Buffer Pack.

*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 (rat) 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.

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 (rat) 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. Do not store diluted antibody.

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

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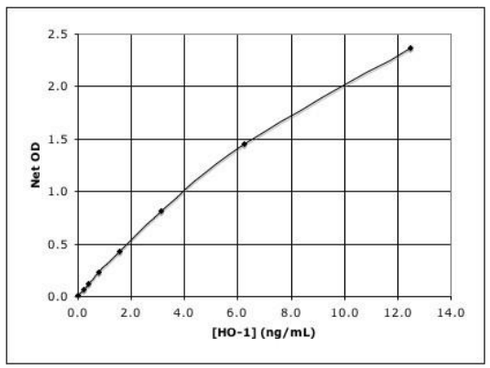
1. Pipet 100 µL of Assay Buffer into the control (0 ng/mL standard) wells.
2. Pipet 100 µL of standards and samples, prepared in Assay Buffer, to the bottom of the appropriate wells.
3. Seal the plate. Incubate for 1 hour at room temperature.
4. Empty the contents of the wells and wash by adding 400 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 0.039 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 HO-1 in cell lysates, serum, EDTA plasma, and microsomes of rat origin. Cross reactivity with rat HO-2 is less than 0.02% and there is no cross reactivity with human and mouse HO-1.

Dilutional Linearity

To determine possible interference from the sample matrix, the indicated sample types were serially diluted into assay buffer. The concentrations of rat 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	C6 CL	S††	EDTA p††	Liver MS
Neat	89%*	62%†	62%†	88%**
1:2	93%	72%	70%	93%
1:4	100%	90%	86%	94%
1:8	---	94%	88%	---
1:16	---	102%	88%	----
1:32	---	105%	90%	---

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

*Cell lysate was diluted 1:60 in Assay Buffer for levels to be within the dynamic range of the assay.

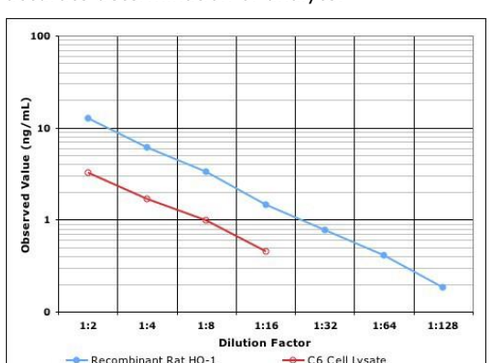
**Microsomes were diluted 1:10 in Assay Buffer for levels to be within the dynamic range of the assay.

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

††Serum and EDTA plasma samples were spiked with recombinant standard.

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.

Reagent	Quantity
ImmunoSet® Buffer Pack	1 of each, total of 5
ImmunoSet® Plate Pack	5 96-well clear microtiter plates & 5 plate sealers
PBS Concentrate	120 mL
BSA Solution (10%)	50 mL
Tween-20 Solution (10%)	30 mL
RIPA Cell Lysis Buffer 2	100 mL
Wash Buffer Concentrate	100 mL
SA-HRP	12.5 µg/vial

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

Novus Biologicals 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. Novus Biologicals 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.