Northwest
Life Science Specialties, LLC

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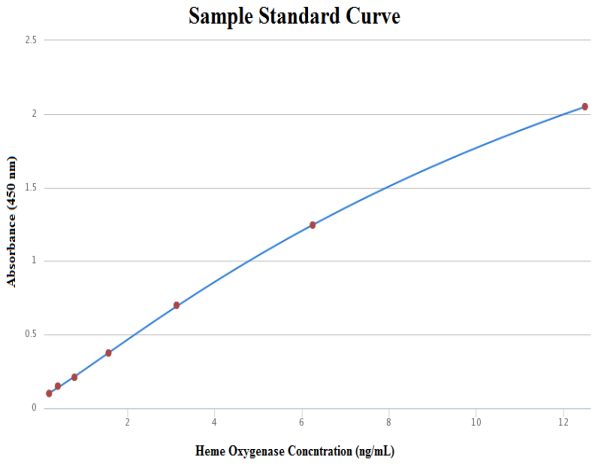
NWLSS™ Rat Heme Oxygenase (HO-1) ELISA

Product NWK-HO1R-01

For Research Use Only



Assay system for measuring the quantity of Rat Heme Oxygenase-1 in plasma, cell lysates and tissue homogenates

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Introduction:

Heme Oxygenase-1 (HO-1) also known as Hsp32, is the inducible isoform of heme oxygenase that catalyzes NADPH, O_2 and Cytochrome P450 reductase dependent oxidation of heme to carbon monoxide, ferrous iron and biliverdin. These products are involved in vasodilation, vascular tone and redox regulation. "Free" iron can increase oxidative stress and regulate the expression of many mRNAs by affecting the conformation of iron regulatory protein (IRP-1) and subsequent binding to iron regulatory elements (IREs). Three HO isoforms catalyzing heme into biliverdin and carbon monoxide have been identified: Inducible HO-1 (Hsp32), constitutive HO-2 abundant in brain and testis and HO-3 which is related to HO-2 but not of the same gene origin. HO activity decreases the levels of heme which is a catalyst for lipid peroxidation and oxygen radical formation. Expression of HO-1 is responsive to all types of oxidative stress related stimuli and it is up-regulated during exposure to oxidants, UV-A irradiation and a series of agents including heme, cytokines, hormones and heavy metals. Oxidative stress has been identified as a potential cornerstone in relation to neurodegenerative diseases such as Alzheimer's (AD), Parkinson's and ALS. HO-1 has been shown to play a role in neuronal defense of oxidative stress related events including heat shock and ischemia. Studies have shown that normal expression of HO-1 in the brain is typically low but increases after a heat shock or ischemic event. Additionally, spatial distribution of HO-1 in AD brains correlates with pathogenic changes in tau proteins associated with neurofibrillary tangles, a hallmark of AD brain lesions.

Intended Use

The NWLSS™ Rat HO-1 ELISA kit provides a simple method to detect and quantify Heme Oxygenase (HO-1) in samples of rat origin. The assay can be used with plasma, cell lysates and tissue homogenates. The assay does not cross react with the other known HO isoforms, HO-2 or HO-3.

Test Principle:

The rat HO-1 ELISA kit is a quantitative sandwich immunoassay. Murine monoclonal antibody specific for HO-1 is pre-coated on the wells of the plate provided. Sample and Standard HO-1 is captured by the stationary plate bound antibody. Captured HO-1 is then reacted with a polyclonal antibody specific for HO-1. The bound antibody-HO-1 complex is detected using an HRP -TMB system. First, anti-rabbit IgG antibody conjugated to horseradish peroxidase (HRP Conjugate) is added and allowed to react. After washing, tetramethylbenzidine (TMB) substrate is then added resulting in blue color development proportional to the amount of HO-1 present in each well. Color development is stopped using an acid stop solution changing the color to yellow which is measured and recorded at 450 nm. HO-1 concentrations in samples are measured by comparing sample 450 nm OD readings with the standard curve.

General Specifications:

Format:	96 well colorimetric
Number of tests:	Triplicate = 24 Duplicate= 40
Specificity:	Rat Heme Oxygenase-1 (HO-1).
Crossreactivity:	Does not crossreact with rat HO-2 or HO-3. Not recommended for measuring human or mouse HO-1. Human HO-1 can be measured using our product NWK-H01H-01.
Sensitivity:	0.036 ng/mL
Effective Range:	0.39 - 12.5ng/mL

Kit Contents

Concentrated Rat HO-1 Standard (5 µg/mL)	1 X 25 µL
5X Extraction Reagent (for cell & tissue homogenates)	1 X 10 mL
Sample/Standard Diluent	1 X 50 mL
20X Wash Buffer	1 X 100 mL
Secondary Antibody (rabbit anti rat HO-1)	1 X 10 mL
HRP Reagent (anti-rabbit IgG/HRP Conjugate)	1 X 10 mL
TMB Substrate	1 X 10 mL
Stop Solution	1 X 10 mL
12 X 8 well strips coated w/ mouse anti rat HO-1)	1 X 96 wells

Required Materials Not Provided:

Adjustable micropipettes with disposable tips (50-1000 µL). Multi-channel pipettes are useful and help to reduce intra-sample variability.

Serological pipettes.

Deionized water.

Polypropylene tubes

Automatic plate washer or other aspiration devices are optional.

Required Instrumentation:

Microtiter plate reader with 450 nm capability.

Warnings, Limitations, Precautions:
Individual components may be harmful if swallowed, inhaled or absorbed through the skin. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact with skin or eyes occurs, rinse the site immediately with water and consult a physician.

Storage & Stability:
Store all components except HO-1 Standard at 4°C until immediately before use. Freeze HO-1 Standard at -20°C until use. All components in this assay are stable for 1 year when stored at 4°C (refrigerated) or frozen at -20°C (concentrated HO-1 Standard only) as specified.

Assay Preparation
1. Determine the number of wells required to assay standards, samples and controls for the appropriate replicate. It is recommended that testing be performed in duplicate or triplicate if possible.

2. Create an assay template showing positioning of standards, controls and samples.

3. Bring all samples and reagents to room temperature before use.

4. To avoid condensation, do not open pouches containing the microtiter strips until after they have reached room temperature. Next remove the required number of strips and place in the frame supplied. Return unused wells to the storage bag with desiccant, seal and store at 2-8ºC.

Reagent Preparation:
1. 20X Wash Buffer
Bring the **20X Wash Buffer** to room temperature and swirl gently to dissolve any crystals that may have formed from storage. Dilute the 50mL of **20X Wash Buffer** in 950 mL deionized water. Label as **Working Wash Buffer.**

Note: Working Wash Buffer is stable at room temperature for up to 4 weeks. For longer-term storage, the Wash Buffer should be stored at 4˚C.

***NOTE**: If not assaying the complete plate, lesser amounts of wash buffer may be diluted as necessary.*

2. Extraction Reagent
Add 10 mL *5X Extraction Reagent* to 40 mL deionized water. Label as **Working Extraction Reagent**. This reagent can be used for processing of cell lysates and tissue homogenates. If the full 50 mL will not be needed mix only what is necessary.

Reagent Preparation (continued):
3. Secondary Antibody, HRP Conjugate, TMB Substrate and Stop Solution
Supplied ready to use.

Calibrator Preparation:
1. Centrifuge the **Concentrated HO-1 Standard** vial before removing the cap to ensure maximal product recovery.

2. Label microtubes 1-7. Add 2.0 mL **Standard/Sample Diluent** to tube 1 and 500 mL Standard/Sample Dilution Buffer to all other tubes.

3. Transfer 5 µL of **HO-1 Standard** (5µg/mL) to tube 1 and mix well to create 12.5 ng/mL standard. Transfer 500 µL from tube 1 to tube 2 and make a 1/2 serial dilution across tubes 2-6 creating standards of 6.25 - 0.39 ng/mL. Leave tube 7 as a diluent buffer only zero control.

Std Tube # :	***1***	***2***	***3***	***4***	***5***	***6***	***7***
Conc. (ng/mL):	12.5	6.25	3.13	1.56	0.78	0.39	0

Note: Diluted Standards should not be stored for future use.

Sample Handling/Preparation
Plasma
Recommended dilution of ETDA rat plasma samples is 1:5.

Cell Lysates 1.
Harvest cells and centrifuge to form a cell pellet. Dump off excess media and resuspend the cells in Working Extraction Buffer as follows:

1 mL of Working Extraction Buffer plus optional protease inhibitors (see below) per 1 million to 10 million cells.

2. Resuspend the cell pellet in the appropriate amount Working Extraction Buffer. Pipette up and down to break up the pellet until the solution is homogeneous.

3. Incubate 30 minutes on ice. If necessary, low level sonication also is acceptable.

4. Centrifuge at high speed (refrigerated) and harvest the clarified supernatant for assay.

Sample Handling/Preparation (continued):

Tissue Homogenates

1. Harvest tissue and process in Working Extraction Buffer using a dounce or similar type homogenizer. Use Working Extraction Buffer as follows:

1 mL of Working Extraction Buffer plus optional protease inhibitors (see below) per 0.5 cm^3 of tissue to be processed.

2. Incubate the tissue homogenate 30 minutes on ice.

3. Transfer homogenate to a suitable tube and centrifuge at high speed (refrigerated) and harvest the clarified supernatant for assay.

Assay Protocol:

1. Bring reagents to room temperature.

2. Prepare HO-1 Standards and samples in Sample Diluent.

3. Add 100µL prepared standards and samples to appropriate wells.

4. Cover the plate and incubate plate at room temperature for 30 minutes.

5. Wash wells with 6 times with 300 µL of 1X Wash Buffer.

6. Add 100µL Secondary Antibody to each well.

7. Cover the plate and incubate plate at room temperature for 1 hour at room temperature.

8. Wash wells with 6 times with 300 µL of 1X Wash Buffer.

9. Add 100µL HRP Conjugate to each well except blanks.

10. Cover the plate and incubate plate at room temperature for 30 minutes.

11. Wash wells 6X with 1X Wash Buffer.

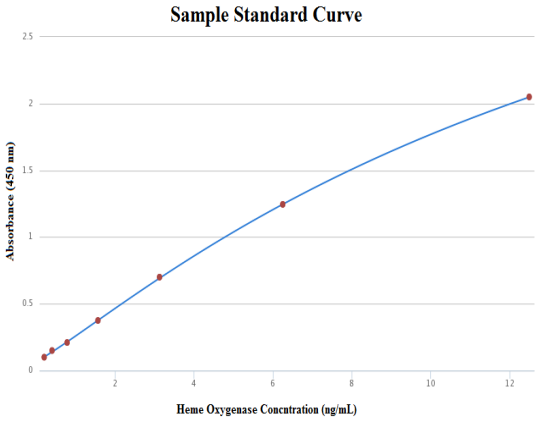
12. Add 100µL TMB Substrate to each well.

13. Incubate at room temperature for 15 minutes in the dark.

14. Add 100µL Stop Solution to each well.

15. Measure absorbance at 450nm.

Data Analysis
Plot the HO-1 standard curve and calculate HO-1 sample concentrations.



Performance Details:
Precision
Intra-Assay (Within Run)
To determine Intra-Assay Precision, three samples of known concentration were assayed thirty times on one plate. The Intra-Assay Coefficient of variation of The HO-1 (human), EIA kit has been determined to be <4%.

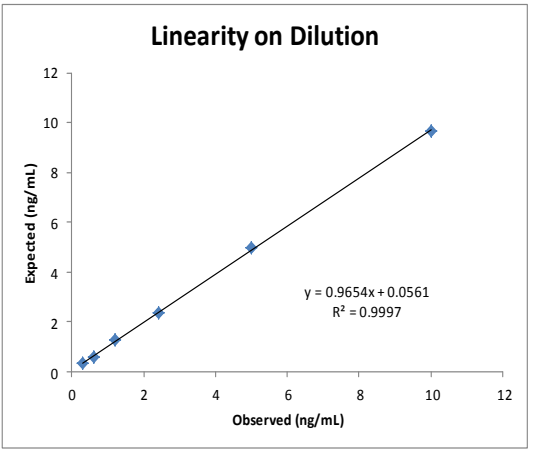
Inter-Assay (Across Plates)
To determine Inter-Assay Precision, three samples of known concentration were assayed thirty times in three individual assays. The Inter-Assay Coefficient of variation of the HO-1 (human) EIA kit has been determined to be <10%.

Sensitivity:
Sensitivity was estimated as 3.29 times the standard deviation for zero HO-1 value. *LLD = 0.036 ng/mL.*

Performance Details (continued)

Accuracy:

Linearity on dilution was tested for a 10 ng/mL rat HO-1 sample in buffer. The sample showed excellent linearity on dilution with an average % difference of 3.36% over the full range.



Recovery on dilution was then tested for various matrices including serum, plasma, cell lysate and tissue homogenate. Average recovery was found to be >90% for each matrix tested

References:

1. Maines, M.D. (1988) FASEB J. 2: 2557-2568.

2. Trakshel, G.M., Kutty, R.K., and Maines, M.D. (1986) J. Biol. Chem. 261: 11131-11137.

3. Maines, M.D. (1997) Cell Mol. Biol. 46: 573-585.

4. Keyse, S.M. and Tyrrell, R.M. (1987) J. Biol. Chem. 268: 14821-14825.

5. Chen, K., Gunter, K. and Mahin, M.D. (2000) J. Neurochem.75: 304-313.

6. Ewing, J.F.and Maines, M.D. (1993) J. Neurochem. 60: 1512-1519.

7. Takeda, A., Onodera, H., Sugimoto, A., Itoyana, Y., Kogure, K. and Shibahara, S. (1994) Brain Res. 666: 120-124.

8. Takeda, A., Smith, M. A., Avila, J. Nunomura, A., Siedlak, S. L., Zhu, X., Perry, G. and Sayre, L.M. (2000) J. Neurochem. 75: 1234-1241.

9. Takeda, A., Perry, G., Abraham, N.G., Dwyer, B.E., Kutty, R.K., Laitinen, J.T., Petersen, R.B. and Smith, M.A. (2000) J. Biol. Chem. 275: 5395-5399.

10. Schipper, H.M., Chertkow, H., Mehindate, K., Frankel, D., Melmed, C. and Bergman, H. (2000) Neurology 54: 1297-1304.

11. Yet, S-F., Tian, R., Layne, M.D., Wang, Z. Y. Maemura, K., Solovyeva, M., Ith, B., Melo, L.G., Zhang, L., Ingwall, J.S., Dzan, V. J., Lee, M-E. and Perella, M.A. (2001) Cir. Res. 89: 168-173.

12. Chen, Y.H., Yet, S.F. and Perrella, M.A. (2003) Exp. Biol. Med. 228: 447-453.

13. Maines, M.D., Raju, V.S. and Panahian, N. (1999) J. Pharmacol. Exp. Ther. 291: 911-919.

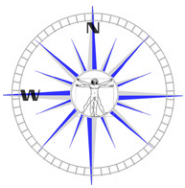
14. Katori, M., Busuttil, R.W. and Kupiec-Weglinski, J.W. (2002) Transplantation 74: 905-912.

14. Lu, F., Zander, D.S. and Visner, G.A. (2002) J. Heart Lung Transplant 21: 1120-1126.

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

User Notes:



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