Datasheet for ABIN2964833

HMOX1 ELISA Kit

3 Images

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Overview

Quantity:	96 tests
Target:	HMOX1
Reactivity:	Human, Rat
Method Type:	Sandwich ELISA
Detection Range:	0.781 ng/mL - 50 ng/mL
Minimum Detection Limit:	0.781 ng/mL
Application:	ELISA

Product Details

Purpose:	Colorimetric detection of heme oxygenase 1
Sample Type:	Cell Lysate, Cyst Fluid, Serum, Tissue Samples
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	0.21 ng/mL
Characteristics:	ELISA kit used to quantitate HO-1 concentration in samples.
Components:	• Anti-HO-1 Immunoassay Plate • 5X HO-1 Extraction Reagent • Recombinant HO-1 Standard • Standard and Sample Diluent • 10X Wash Buffer Concentrate • Anti-HO-1 Biotinylated Antibody Concentrate

Product Details

	• Anti-HO-1 Biotinylated Antibody Diluent • Streptavidin: HRP Concentrate • Streptavidin: HRP Diluent • TMB Substrate • Stop Solution • Pre-treatment Buffer
Material not included:	- Ultra pure water - Additional reagents and materials for cell lysate and tissue extract preparation, including protease inhibitors - Precision pipettors, with disposable plastic tips - Polypropylene or polyethylene tubes to prepare samples – do not use polystyrene, polycarbonate or glass tubes - A container to prepare 1X Wash Buffer - A wash bottle or an automated 96-well plate washer

Target Details

Target:	HMOX1
Alternative Name:	HO-1 (HMOX1 Products)
Background:	Heme-oxygenase is a ubiquitous enzyme that catalyzes the initial and rate-limiting steps in heme catabolism yielding equimolar amounts of biliverdin, iron and carbon monoxide. Biliverdin is subsequently converted to bilirubin and the free iron is sequestered to ferritin. These products have important physiological effects as carbon monoxide is a potent vasodilator, biliverdin and bilirubin are potent antioxidants, and the free iron increases oxidative stress and regulates the expression of many mRNAs. There are three isoforms of heme-oxygenase, HO-1, HO-2 and HO-3, however HO-1 and HO-2 are the major isoforms as they both have been identified in mammals. HO-1, also known as heat shock protein 32, is an inducible isoform activated by most oxidative stress inducers, cytokines, inflammatory agents and heat shock. HO-2 is a constitutive isoform which is expressed under homeostatic conditions. HO-1 is also considered to be a cytoprotective factor in that free heme is highly reactive and cytotoxic, and secondly, carbon monoxide is a mediator inhibiting the inflammatory process and bilirubin is a scavenger for reactive oxygen, both of which are the end products of heme catalyzation. It has also been shown that HO-1 deficiency may cause reduced stress defense, a pro-inflammatory tendency, susceptibility to atherosclerotic lesion formation, endothelial cell injury, and growth retardation. Up-regulation of HO-1 is therefore said to be one of the major defense mechanisms of oxidative stress.

Target Details

Pathways:	Transition Metal Ion Homeostasis, Regulation of Leukocyte Mediated Immunity, Positive Regulation of Immune Effector Process, Production of Molecular Mediator of Immune Response, SARS-CoV-2 Protein Interactome

Application Details

Assay Time:	0.5 h
Plate:	Pre-coated
Protocol:	1. Prepare Standard and samples in Standard and Sample Diluent. 2. Add 50 µL of Pre-Treatment Buffer to all sample and standard wells. 3. Add 50 µL of Standard and sample to appropriate wells. 4. Cover plate with Plate Sealer and incubate at room temperature (20-25 °C) for 2 hours. 5. Wash plate four times with 1X Wash Buffer. 6. Add 100 µL of Biotinylated Antibody Working Solution to each well. 7. Cover plate with Plate Sealer and incubate at room temperature for 1 hour. 8. Wash plate four times with 1X Wash Buffer as described in step 9. 9. Add 100 µL of Streptavidin-HRP Working Solution to each well. 10. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes. 11. Wash plate four times with 1X Wash Buffer as described in step 12. 12. Add 100 µL of TMB Substrate to each well. 13. Develop the plate in the dark at room temperature for 30 minutes. 14. Stop reaction by adding 100 µL of Stop Solution to each well. 15. Measure absorbance on a plate reader at 450 nm.
Assay Procedure:	1. Prepare Standard and samples in Standard and Sample Diluent. 2. Add 50 µL of Pre-Treatment Buffer to all sample and standard wells. 3. Add 50 µL of Standard and sample to appropriate wells. 4. Cover plate with Plate Sealer and incubate at room temperature (20-25 °C) for 2 hours. 5. Wash plate four times with 1X Wash Buffer. 6. Add 100 µL of Biotinylated Antibody Working Solution to each well. 7. Cover plate with Plate Sealer and incubate at room temperature for 1 hour. 8. Wash plate four times with 1X Wash Buffer as described in step 9. 9. Add 100 µL of Streptavidin-HRP Working Solution to each well. 10. Cover plate with Plate Sealer and incubate at room temperature for 30 minutes. 11. Wash plate four times with 1X Wash Buffer as described in step 12. 12. Add 100 µL of TMB Substrate to each well. 13. Develop the plate in the dark at room temperature for 30 minutes. 14. Stop reaction by adding 100 µL of Stop Solution to each well. 15. Measure absorbance on a plate reader at 450 nm.
Calculation of Results:	Duplicate absorbance values should be within 10% of each other. Care should be taken when interpreting data with differences in absorbance values greater than 10%.

Application Details

1. Prepare a standard curve to determine the amount of HO-1 in an unknown sample. Plot the average absorbance obtained for each standard concentration on the vertical (Y) axis versus the corresponding HO-1 concentration on the horizontal (X) axis using graph paper or curve-fitting software.

2. Calculate the HO-1 concentration in unknown samples using the prepared standard curve. Determine the amount of HO-1 in each unknown sample by noting the HO-1 concentration (X axis) that correlates with the absorbance value (Y axis) obtained for the unknown sample.

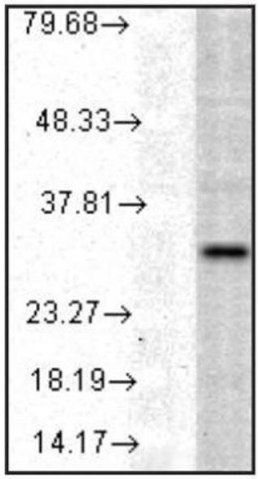
3. Multiply the HO-1 concentration obtained by the dilution factor to determine the amount of HO-1 in the undiluted sample.

Restrictions:	For Research Use only

Handling

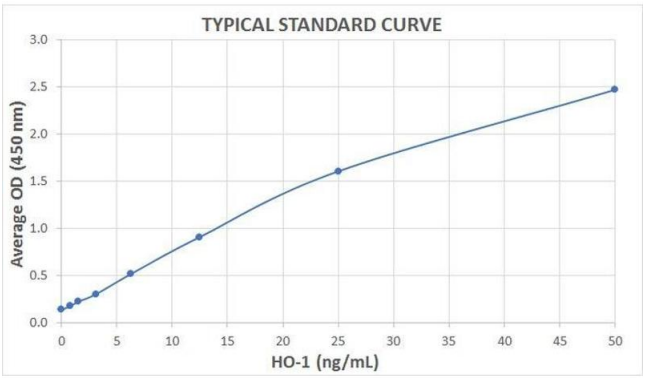
Storage:	4 °C

Images



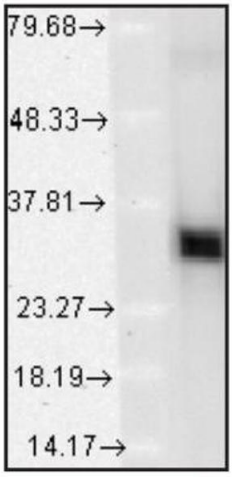
Western Blotting

Image 1. VAlidation of Detection Antibody: Western blot analysis of HO-1 in a human cell line mix showing specificity at ~32kDa



ELISA

Image 2. Typical Standard Curve for the HO-1 ELISA Kit (Enzyme-Linked Immunosorbent Assay). Assay Type: Sandwich ELISA. Detection Method: Colorimetric Assay. Assay Range: 0.781 – 50 ng/mL.



Western Blotting

Image 3. Validation of Capture Antibody: Western blot analysis of HO-1 in mouse tissues showing absolute specificity at ~32kD