KAMIYA BIOMEDICAL COMPANY

Human HO-1/HMOX1 ELISA

For the quantitative determination of HMOX1 in human cell culture supernates, cell lysates, serum and plasma (heparin, EDTA)

Cat. No. KT-1298

For Research Use Only. Not for diagnostic use in the U.S.

PRODUCT INFORMATION

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INTENDED USE
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INTRODUCTION
HMOX1 (heme oxygenase (decycling) 1) is a human gene that encodes for the enzyme heme oxygenase 1. It is localized to chromosome 22. Heme oxygenase, an essential enzyme in heme catabolism, cleaves heme to form biliverdin, which is subsequently converted to bilirubin by biliverdin reductase, and carbon monoxide, a putative neurotransmitter. Heme oxygenase activity is induced by its substrate heme and by various nonheme substances. Heme oxygenase occurs as 2 isozymes, an inducible heme oxygenase-1 and a constitutive heme oxygenase-2. HMOX1 and HMOX2 belong to the heme oxygenase family.

PRINCIPLE
The Human HMOX1 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid phase immunoassay specially designed to measure Human HMOX1 with a 96-well strip plate that is pre-coated with antibody specific for HMOX1. The detection antibody is a biotinylated antibody specific for HMOX1. The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human HMOX1 with immunogen: Expression system for calibrator: E.coli; Immunogen sequence: M1-M288. The kit is analytically validated with ready to use reagents.

To measure Human HMOX1, add calibrators and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is substrate to HRP and will be catalyzed to produce a blue color product, which changes into yellow after adding acidic stop solution. The density of the yellow product is linearly propotional to Human HMOX1 in the sample. Read the density of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the calibration curve to determine the concentration of Human HMOX1 in the sample.

COMPONENTS
1. Anti-Human HMOX1 Pre-coated 96-well strip microplate: 1 (12 strips of 8 wells)

2. Human HMOX1 Calibrator: 10 ng/tube x 2

3. Human HMOX1 Biotinylated antibody (100X): 130 µL

4. Avidin-Biotin-Peroxidase Complex (100X): 130 µL

5. Sample Diluent: 30 mL

6. Antibody Diluent: 12 mL

7. Avidin-Biotin-Peroxidase Diluent: 12 mL

8. Color Developing Reagent (TMB): 10 mL

9. Stop Solution: 10 mL

10. Plate Sealers: 4

11. Wash Buffer Concentrate: Powder for 1,000 mL

MATERIALS REQUIRED BUT NOT PROVIDED

Microplate Reader capable of reading absorbance at 450 nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500 mL graduated cylinders.
Test tubes for dilution.

REAGENT PREPARATION

All reagents: Bring all reagents to 37℃ prior to use. The assay can also be done at room temperature however we recommend doing it at 37℃ for best consistency with our QC results. Also the TMB incubation time estimate (15-20 min) is based on 37℃.

Wash buffer: Dissolve the included powder in 1,000 mL of deionized water. Excess wash buffer can be stored for up to one week at 4℃.

Biotinylated Anti-Human HMOX1 antibody: It is recommended to prepare this reagent immediately prior to use by diluting the Human HMOX1 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µL by adding 1 µL of Biotinylated antibody (100x) to 99 µL of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Avidin-Biotin-Peroxidase Complex: It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µL by adding 1 µL of Avidin-Biotin-Peroxidase Complex (100x) to 99 µL of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.

Human HMOX1 Calibrator: It is recommended that the calibrators be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human HMOX1 calibrator for each experiment. Gently spin the vial prior to use. Reconstitute the calibrator to a stock concentration of 10 ng/mL using 1 mL of sample diluent. Allow the calibrator to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.

Microplate: The included microplate is coated with capture antibodies and ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Dilution of Human HMOX1 Calibrator

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –6,000 pg/mL, #2 –3,000 pg/mL, #3 – 1,500 pg/mL, #4 – 750 pg/mL, #5 – 375 pg/mL, #6 – 187.5 pg/mL, #7 – 93.75 pg/mL, #8 – 0.0 (Blank).
2. To generate calibrator #1, add 600 µL of the reconstituted calibrator stock solution of 10 ng/mL and 400 µL of sample diluent to tube #1 for a final volume of 1,000 µL. Mix thoroughly.
3. Add 300 µL of sample diluent to tubes # 2-7.
4. To generate calibrator #2, add 300 µL of calibrator #1 from tube #1 to tube #2 for a final volume of 600 µL. Mix thoroughly.
5. To generate calibrator #3, add 300 µL of calibrator #2 from tube #2 to tube #3 for a final volume of 600 µL. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank calibrator to be used with every experiment.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Cell culture supernatants: Clear sample of particulates by centrifugation, assay immediately or store samples at -20℃.

Serum: Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20℃.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20℃.
*Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.

Cell lysates: Lyse the cells, make sure there are no visible cell sediments. Centrifuge cell lysates at approximately 10,000 X g for 5 min. Collect the supernatant.

Sample Dilution
The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

It is recommended to prepare 150 µL of sample for each replicate to be assayed. The samples should be diluted with sample diluent and mixed gently.

ASSAY PROTOCOL

It is recommended that all reagents and materials be equilibrated to 37℃/room temperature prior to the experiment (see Reagent Preparation if you have missed this information).
1. Prepare all reagents and working calibrators as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µL of the calibrator, samples, or control per well. Add 100 µL of the sample diluent buffer into the control well (Zero well). At least two replicates of each calibrator, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37℃).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µL of the prepared 1x Biotinylated Anti-Human HMOX1 antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37℃).
8. Wash the plate 3 times with the 1x wash buffer.
a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
c. Repeat steps a-b 2 additional times.
9. Add 100 µL of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37℃).
10. Wash the plate 5 times with the 1x wash buffer.
a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
b. Add 300 µL of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
c. Repeat steps a-b 4 additional times.
11. Add 90 µL of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 25-30 minutes at 37℃). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four calibrator wells, while the remaining calibrators remain clear.)
12. Add 100 µL of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

DATA ANALYSIS

Average the duplicate readings for each calibrator, sample, and control. Subtract the average zero calibrator O.D. reading.
It is recommended that a calibration curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay.

Alternatively, plot the mean absorbance for each calibrator against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the calibration curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the calibration curve must be multiplied by the dilution factor.

OVERVIEW

Product Name Human HO-1/HMOX1 ELISA
Reactive Species Human
Size 96 wells/kit, with removable strips.
Description Sandwich High Sensitivity ELISA kit for Quantitative Detection of Human HMOX1. 96 wells/kit, with removable strips.
Sensitivity <10 pg/mL
*The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range 93.7 pg/mL-6,000 pg/mL
Storage Instructions Store at 4℃ for 6 months, at -20℃ for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID P09601

TECHNICAL DETAILS

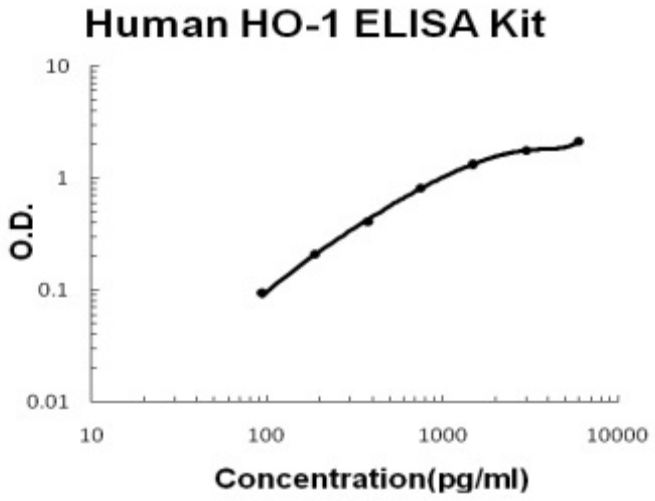
Capture/Detection Antibodies The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.
Specificity Natural and recombinant Human HMOX1
Immunogen Expression system for calibrator: E.coli; Immunogen sequence: M1-M288
Cross Reactivity There is no detectable cross-reactivity with other relevant proteins.

EXAMPLE CALIBRATION CURVE

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/mL)	0	93.7	187.5	375	750	1,500	3,000	6,000
O.D.	0.010	0.095	0.210	0.407	0.814	1.333	1.763	2.133

Human HMOX1 ELISA Kit calibration curve



A calibration curve is provided for demonstration only. A calibration curve should be generated for each set of samples assayed.

INTRA/INTER ASSAY VARIABILITY

We spend great efforts in documenting lot to lot variability and making sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision across assays): Three samples of known concentration were tested in separate assays to assess inter-assay precision.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	149	658	3760	138	610	3391
Standard deviation	11.62	32.9	180.48	12.69	39.65	200.06
CV(%)	7.8%	5%	4.8%	9.2%	6.5%	5.9%

REPRODUCIBILITY

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	Lot1 (pg/ml)	Lot2 (pg/ml)	Lot3 (pg/ml)	Lot4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	149	156	130	134	142	10.63	7.4%
Sample 2	658	603	678	602	635	33.5	5.2%
Sample 3	3760	3562	3498	3701	3630	104.86	2.8%

*number of samples for each test n=16.

PRECAUTION

Please read the following instructions before starting the experiment.
1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using calibrators and a small number of samples is recommended.
2. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
3. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
4. Don't reuse tips and tubes to avoid cross contamination.
5. Avoid using the reagents from different batches together.

FOR RESEARCH USE ONLY

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