ab133064 – HO-1 Human ELISA Kit

Instructions for Use

For quantitative detection and quantitation of HO-1 in cell lysates and tissue extracts.

This product is for research use only and is not intended for diagnostic use.

Version 4 Last Updated 15 January 2019

Table of Contents

INTRODUCTION

1. BACKGROUND 2
2. ASSAY SUMMARY 5

GENERAL INFORMATION

3. PRECAUTIONS 6
4. STORAGE AND STABILITY 7
5. MATERIALS SUPPLIED 7
6. MATERIALS REQUIRED, NOT SUPPLIED 8
7. LIMITATIONS 8
8. TECHNICAL HINTS 9

ASSAY PREPARATION

9. REAGENT PREPARATION 10
10. STANDARD PREPARATIONS 11
11. SAMPLE COLLECTION AND STORAGE 13
12. PLATE PREPARATION 15

ASSAY PROCEDURE

13. ASSAY PROCEDURE 16

DATA ANALYSIS

14. CALCULATIONS 18
15. TYPICAL DATA 19
16. TYPICAL SAMPLE VALUES 20
17. ASSAY SPECIFICITY 21

RESOURCES

18. TROUBLESHOOTING 22
19. NOTES 23

1. BACKGROUND

Abcam's HO-1 *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of HO-1 in cell lysates and tissue extracts.

A mouse monoclonal antibody specific for HO-1 is pre-coated on the wells of a plate. Standards or test samples are added to the wells, incubated and then washed. A HO-1 polyclonal antibody is then added, incubated and washed. An HRP conjugated anti-IgG antibody is then added, incubated. The plate is washed once more and the TMB substrate is then added which HRP catalyzes, generating a blue coloration after incubation. A stop solution is added which generates conversion to yellow color read at 450 nm which is proportional to the amount of analyte bound.

Heme Oxygenase-1 (HO-1) also known as Hsp32, is the inducible isoform of heme oxygenase that catalyzes the NADPH, O2 and cytochrome P450 reductase dependent oxidation of heme to carbon monoxide, ferrous iron and biliverdin which is rapidly reduced to bilirubin. These products of the HO reaction have important physiological effects: carbon monoxide is a potent vasodilator and has been implicated to be a physiological regulator of cGMP and vascular tone; biliverdin and its product bilirubin are potent antioxidants; "free" iron increases oxidative stress and regulates the expression of many mRNAs (e.g., DCT-1, ferritin and transferring receptor) by affecting the conformation of iron regulatory protein (IRP)-1 and its binding to iron regulatory elements (IREs) in the 5'- or 3'- UTRs of the mRNAs. To date, three identified heme oxygenase isoforms are part of the HO system that catalyze heme into biliverdin and carbon monoxide. These are inducible HO-1 or Hsp32, constitutive HO-2 that is abundant in the brain and testis, and HO-3 which is related to HO-2 but is the product of a different gene. The HO system is the rate-limiting step in heme degradation and HO activity decreases the levels of heme which is a well known potent catalyst of lipid peroxidation and oxygen radical

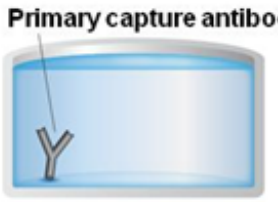
formation. The expression of HO-1 is highly responsive to all types of stimuli that cause oxidative stress and it is up regulated during exposure to oxidants, UV-A irradiation and a series of agents including cytokines, hormones, heme and heavy metals. HO-1 is a vital component of neuronal defense mechanisms and oxidative stress has been postulated to be the underlying basis for neuronal cell death in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease. The expression of HO-1 is normally very low in the brain but increases markedly after heat shock, ischemia or glutathione depletion. Spatial distribution of HO-1 expression in AD brain is essentially identical to that of the pathogenic conformational changes of tau protein that is the major component of the intraneuronal lesion of AD, neurofibrillary tangles.

HO-1 expression and tau expression may be regulated by oxidative stresses in a coordinated manner and play a pivotal role in the cytoprotection of neuronal cells9. Plasma and cerebrospinal fluid HO-1 protein and lymphocyte HO-1 mRNA levels are decreased in subjects with sporadic AD relative to normal elderly controls suggesting that measurement of HO-1 may serve as a useful biological marker in early sporadic AD.

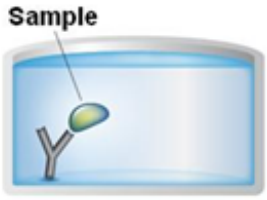
Oxidative stress in the heart caused by ischemia and reperfusion has been shown to lead to cardiomyocyte death. An absence of HO-1 has detrimental consequences whereas over expression of HO-1 plays a protective role in hypoperfusion and ischemia/reperfusion-induced myocardial injury. Under normal conditions, HO-1 is present at low levels in all organs except the spleen, but its expression is rapidly accelerated in response to pathophysiological conditions such as renal ischemia/reperfusion and cellular transformation. HO-1 over expression exerts beneficial cytoprotective effects in a number of transplantation models, including antigen-independent ischemia/reperfusion injury, acute and chronic allograft rejection and xenotransplantation.

The mechanisms by which HO-1 confers its protective effects are currently poorly understood but this area of investigation is active and rapidly evolving. The measurement of HO-1 in various cell types, tissues and bodily fluids may provide new insights into the physiological roles of HO-1 and may lead to monitoring HO-1 levels as a biomarker for therapeutic interventions or as an environmental biomarker in toxicology studies.

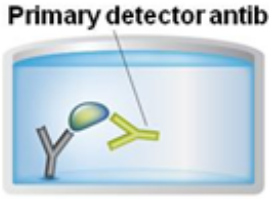
2. ASSAY SUMMARY



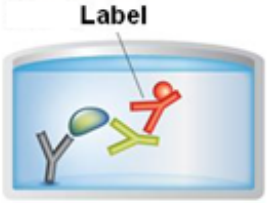
Prepare all reagents, samples and standards as instructed.



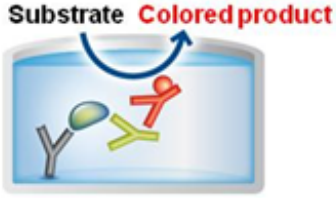
Add standard or sample to each well used. Incubate at room temperature.



Wash and add antibody to each well. Incubate at room temperature.



Wash and add prepared Antibody-HRP Conjugate. Incubate at room temperature.



Add TMB Substrate to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

- Stop Solution 2 is a 1 normal (1N) hydrochloric acid solution. This solution is caustic; care should be taken in use
- The activity of the Horseradish peroxidase conjugate is affected by nucleophiles such as azide, cyanide and hydroxylamine
- We test this kit's performance with a variety of samples, however it is possible that high levels of interfering substances may cause variation in assay results

4. STORAGE AND STABILITY

Store kit at 4°C immediately upon receipt, apart from the HO-1 Standard, which should be stored at -20°C. Avoid multiple freeze-thaw cycles.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
Microplate coated with monoclonal anti-HO-1 antibody (12 x 8 wells)	96 Wells	4ºC
Anti-rabbit IgG HRP Conjugate	25 µL	4ºC
Sample Diluent	50 mL	4ºC
5X Extraction Reagent	10 mL	4ºC
HRP Conjugate Diluent	11 mL	4ºC
Antibody Diluent	11 mL	4ºC
Recombinant HO-1 Standard	25 µL	-20ºC
20X Wash Buffer Concentrate	100 mL	4ºC
TMB Substrate	10 mL	4ºC
Stop Solution 2	10 mL	4ºC
Anti-Human HO-1	25 µL	4ºC

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Standard microplate reader - capable of reading at 450 nm, preferably with correction between 570 and 590 nm
- Automated plate washer (optional)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed
- Eppendorf tubes
- Microplate Shaker
- Absorbent paper for blotting
- Deionized water

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted

8. TECHNICAL HINTS

- Standards can be made up in either glass or plastic tubes
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample, standard and reagent
- Pipette standards and samples to the bottom of the wells
- Add the reagents to the side of the well to avoid contamination
- This kit uses break-apart microtiter strips, which allow the user to measure as many samples as desired. Unused wells must be kept desiccated at 4°C in the sealed bag provided. The wells should be used in the frame provided
- Prior to addition of substrate, ensure that there is no residual wash buffer in the wells. Any remaining wash buffer may cause variation in assay results
- It is important that the matrix for the standards and samples be as similar as possible. HO-1 samples diluted with Sample diluent should be run with a standard curve diluted in the same buffer. Culture supernatant samples should be read against a standard curve diluted in the same complete but non-conditioned media
- A 5X Extraction Reagent has been included in this assay. Use of other lysis or extraction buffers may interfere with the performance of the assay
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.

9.1 Anti-Rabbit IgG: HRP Conjugate

9.1.1 Centrifuge the vial before removing the cap to ensure maximum product recovery.

9.1.2 Dilute 22µL of HRP Conjugate in 11mL of HRP Conjugate Diluent in a polypropylene tube. If only using a portion of the plate, dilute only what is needed for number of wells used.

9.1.3 Mix gently by inversion.

9.1.4 Reagent is now ready to be used in the Assay Procedure.

9.1.5 Do not re-use or store any remaining diluted HRP Conjugate.

9.2 Anti-Human HO-1

9.2.1 Centrifuge the vial before removing the cap to ensure maximum product recovery.

9.2.2 Dilute 22µL of Anti-HO-1 in 11mL of Anti-HO-1 Diluent in a polypropylene tube. If only using a portion of the plate, dilute only what is needed for number of wells used.

9.2.3 Mix gently by inversion.

9.2.4 Reagent is now ready to be used in the Assay Procedure.

9.2.5 Do not re-use or store any remaining diluted Anti-Human HO-1.

9.3 1X Wash Buffer

Prepare the 20X Wash Buffer by bringing to room temperature and swirl gently to dissolve any crystals that may have formed from storage Dilute 100 mL of the 20X Wash Buffer Concentrate in 1,900 mL of deionized water. Mix thoroughly and gently. Bring the 20X Wash Buffer

10. STANDARD PREPARATIONS

Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use. Diluted standards should be used within 60 minutes of preparation. Centrifuge the standard before removing the cap.

10.1 Allow the reconstituted 5,000 ng/mL HO-1 **Stock Standard** solution to equilibrate to room temperature. The standard solution should be aliquoted, stored at -20°C and subjected to no more than two freeze/thaw cycles. Dilute standards with the appropriate diluent for the samples being analyzed.

10.2 Label seven tubes with numbers 1 – 7.

10.3 Add 250 µL sample diluent to tubes 2– 7.

10.4 Prepare a 25 ng/mL **Standard 1** by adding 5 µL of the 5,000 ng/mL Stock Standard to Add 995 µL of the sample diluent to tube 1. Mix thoroughly and gently.

10.5 Prepare **Standard 2** by transferring 250 µL from Standard 1 to tube 2. Mix thoroughly and gently.

10.6 Prepare **Standard 3** by transferring 250 µL from Standard 2 to tube 3. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes 4 through 6.

10.8 $\mathbf{B_O}$ contains no protein and is the blank control.

Standard	Sample to Dilute	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	Stock	5	995	5,000	25
2	Standard 1	250	250	25	12.5
3	Standard 2	250	250	12.5	6.25
4	Standard 3	250	250	6.25	3.125
5	Standard 4	250	250	3.125	1.56
6	Standard 5	250	250	1.56	0.78
7	None	-	250	-	-

11. SAMPLE COLLECTION AND STORAGE

- This assay is suitable for measuring HO-1 in cell lysates and tissue extracts samples. Citrate and heparin plasma have not been validated for use. Prior to the assay, frozen samples should be slowly brought to 4˚C and centrifuged, if necessary, to remove residual debris. Users must determine the optimal dilutions for their particular experiments

Extraction of samples -

Suggested protocols for the preparation of cell lysates and tissue extracts samples may be found below. Investigators may use alternative methods of cell and tissue lysate preparation, however, it is recommended that the 5X Extraction Reagent provided in this kit be diluted to 1X and used as the lysis buffer. Use of alternative lysis buffers may contain components, which could interfere and compromise the performance of the assay, producing inaccurate results.

11.1 Preparation of cell lysates -

11.1.1 For adherent cell lines, aspirate the media and wash the cells three times with phosphate buffered saline. Harvest the cells using appropriate, established methods (e.g. scraping, trypsinization) and centrifuge cells to pellet.

For non-adherent cell lines, centrifuge cells to pellet, aspirate media and wash cells three times with phosphate buffered saline.

11.1.2 Aspirate the supernatant from the final wash. If necessary, the cell pellet can be frozen at -70°C and processed at a later date.

11.1.3 Calculate the amount of 1X Extraction Reagent that will be required. For every 1 X 106 to 1 X 107 cells, use 1 mL of Extraction Reagent.

11.1.4 Dilute an appropriate amount of 5X Extraction Reagent with cold (4°C) deionized or distilled water to generate the required volume of 1X Extraction Reagent. For example, if 5 mL of 1X Extraction Reagent were required, dilute 1 mL of the 5X Extraction Reagent with 4 mL of cold deionized or distilled water.

11.1.5 Add protease inhibitors to the 1X Extraction Reagent. Examples of appropriate protease inhibitors include 0.1 mM PMSF, 1 µg/mL leupeptin, 1 µg/mL aprotinin, 1 µg/mL pepstatin. Alternatively, a protease inhibitor cocktail tablet can be added at a final 1X concentration.

11.1.6 Resuspend the cell pellet with an appropriate volume of 1X Extraction Reagent supplemented with protease inhibitors. Pipet up and down to break up the cell pellet until the cell suspension is homogeneous and no clumps are visible.

11.1.7 Incubate 30 minutes on ice with occasional mixing or alternatively, samples can be briefly sonicated.

11.1.8 Transfer extracts to polypropylene microcentrifuge tubes and centrifuge at 21,000 x g for 10 minutes in a 4°C refrigerated microfuge.

11.1.9 Transfer the supernatants to labeled polypropylene tubes. When collecting the supernatant, avoid disturbing the cell pellet. The supernatant collected is the cell lysate, which is now ready for analysis using the HO-1 Human ELISA kit. The resulting pellets can be discarded. Alternatively, the cell lysates can be frozen at -70°C and assayed at a later date. It is recommended that a protein assay be performed and the lysates aliquotted to convenient amounts prior to storing at -70°C to avoid multiple freeze thaw cycles.

11.2 Preparation of tissue samples -

1. Harvest tissue to be analyzed. If necessary, tissues can be flash frozen, stored at -70°C and the extract prepared at a later time.

11.2.2 Calculate the amount of 1X Extraction Reagent that will be required. For each ~0.5 cm^3 piece of tissue, use 1 mL of 1X Extraction Reagent.

11.2.3 Dilute an appropriate amount of 5X Extraction Reagent with cold (4°C) deionized or distilled water to generate the required volume of 1X Extraction Reagent. For example, if 5mL of 1X Extraction Reagent were required, dilute 1 mL of the 5X Extraction Reagent with 4 mL of cold deionized or distilled water.

11.2.4 Add protease inhibitors to the Extraction Reagent. Examples of appropriate protease inhibitors include 0.1 mM PMSF, 1 µg/mL leupeptin, 1 µg/mL aprotinin, 1 µg/mL pepstatin. Alternatively, a protease inhibitor cocktail tablet can be added at a final 1X concentration.

11.2.5 Place the tissue in a mortar and add a sufficient volume of liquid nitrogen to cover the tissue.

11.2.6 Allow the liquid nitrogen to evaporate. The tissue should be thoroughly frozen.

11.2.7 Grind the frozen tissue to a powder with a pestle.

11.2.8 Add an appropriate volume of 1X Extraction Reagent supplemented with protease inhibitors to the processed tissue.

11.2.9 Continue to homogenize the tissue with the pestle until the tissue suspension is homogeneous.

11.2.10 Transfer the extract to a polypropylene tube and centrifuge at 21,000 x g for 10 minutes in a 4°C refrigerated microfuge.

11.2.11 Transfer the supernatant to a labeled polypropylene tube. The supernatant collected is the tissue extract, which is now ready for analysis using the HO-1 Human ELISA kit.

The resulting pellet can be discarded. Alternatively, the tissue extracts can be frozen at -70°C and assayed at a later date. It is recommended that a protein determination assay be performed and the extracts aliquotted to convenient amounts prior to storing at -70°C to avoid multiple freeze thaw cycles.

11.3 Chemical Compatibility Limits -

Different chemicals may interfere with the HO-1 (human) EIA kit. Although the effect of every chemical is not known, the following chemicals to determine the level at which they may interfere with the kit.

The compatible limit is defined as the chemical concentration at which the measurement of HO-1 in a sample is inhibited by ≤10%.

Chemical	Compatible Limit
Aprotinin	50 µg/mL
ß-mercaptoethanol	0.75 mM
CHAPS	1% (w/v)
Dithiothreitol (DTT)	0.2 mM
EDTA	5 mM
Glycerol	1% (v/v)
HEPES, pH 7.5	5 mM
Leupeptin	50 µg/mL
Magnesium Chloride (MgCl2)	5 mM
MOPS, pH 7.5	250 mM
NP-40	0.02% (v/v)
Pepstatin A	10 µg/mL
PMSF	0.5 mM
SDS	0.01% (w/v)
Sodium Azide (NaN3)	0.5% (w/v)
Sodium Deoxycholate	0.2% (w/v)
Sodium Chloride (NaCl)	200mM
Sodium Phosphate, pH 7.2	50 mM
Tris, pH 7.5	250 mM
Triton-X100	<0.01% (v/v)
Tween-20	1% (v/v)

11.4 Dilution of samples -

Cell and tissue lysates may be diluted 1/50 (v/v) in Sample Diluent as a suggested starting dilution only. Additional dilutions may be necessary to ensure that sample values are within the range of the standard curve. Users must determine the optimal sample dilutions for their particular experiments.

3. Dilute prepared samples (i.e. cell and tissue lysates) in Sample Diluent. Prepare at least 250 µL of diluted sample to permit assaying in duplicate.

11.4.2 Mix thoroughly.

11.4.3 Samples are now ready to be used in the Assay Procedure Samples may be left at room temperature while Reagents are being prepared.

- Store samples to be assayed within 24 hours at 2 - 8°C. For long-term storage, aliquot and freeze samples at -70°C. Avoid repeated freeze-thaw cycles

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature prior to use**
- **It is recommended to assay all standards, controls and samples in duplicate**

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL of Standards 1 through 7 into the appropriate wells.

13.3 Add 100 µL of the Samples into the appropriate wells.

13.4 Seal the plate and incubate for 30 minutes shaking at room temperature.

13.5 Empty the contents of the wells and wash by adding 300 µL of 1X Wash Buffer to every well. Repeat the wash 5 more times for a total of 6 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.

13.6 Add 100 µL of the HO-1 Antibody to every well.

13.7 Cover wells with a fresh adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour, preferably with gentle mixing.

13.8 Wash plate as described in step 13.5.

13.9 Add 100 µL of HRP conjugate to each well.

13.10 Cover wells with a fresh adhesive plate sealer or plastic wrap and incubate at room temperature for 1 hour, preferably with gentle mixing.

13.11 Wash plate as described in step 13.5.

13.12 Add 100 µL of the TMB Substrate solution to every well. Incubate at room temperature for 15 minutes on a plate shaker.

13.13 Add 100 µL Stop Solution 2 into each well in the same order that the TMB Substrate was added. The plate should be read immediately.

13.14 Read the O.D. absorbance at 450 nm, preferably with correction between 570 and 590 nm.

14. CALCULATIONS

A four parameter algorithm (4PL) provides the best fit, though other equations can be examined to see which provides the most accurate (e.g. linear, semi-log, log/log, 4 parameter logistic). Interpolate protein concentrations for unknown samples from the standard curve plotted. Samples producing signals greater than that of the highest standard should be further diluted and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.

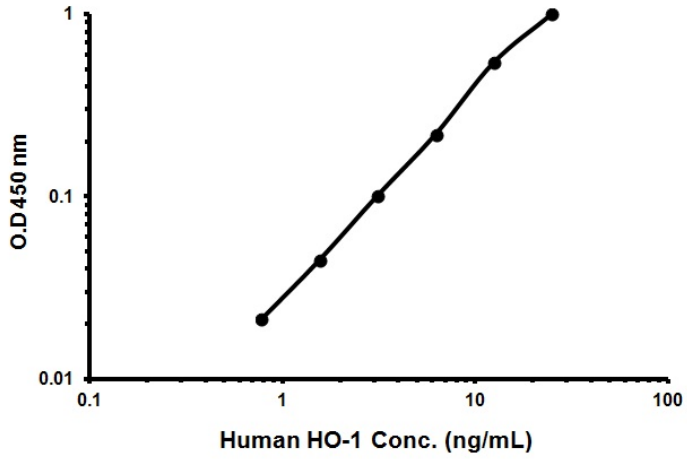
- Calculate the average net Optical Density (OD) bound for each standard and sample by subtracting the average blank control OD from the average OD bound:

  Average Net OD = Average Bound OD – Average blank control OD

- Plot the average Net OD for each standard versus Human p21 concentration in each standard. Sample concentrations may be calculated off of Net OD values using the desired curve fitting

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Sample	Conc. (ng/mL)	Mean O.D. (-Blank)
Standard 1	25	1.000
Standard 2	12.5	0.544
Standard 3	6.25	0.220
Standard 4	3.125	0.102
Standard 5	1.56	0.045
Standard 6	0.78	0.021

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The sensitivity of the HO-1 EIA has been determined to be 0.78 ng/mL. The standard curve has a range of 0.78 to 25 ng/mL.

PRECISION –

Intra-Assay Precision (Within Run Precision)

To determine Intra-Assay Precision, three samples of known concentration were assayed twenty times on one plate. The Intra-Assay Coefficient of variation of the HO-1 EIA has been determined to be <10%.

Inter-Assay Precision (Between Run Precision)

To determine Inter-Assay Precision, three samples of known concentration were assayed multiple times in several individual assays. The Inter-Assay Coefficient of variation of the HO-1 EIA has been determined to be <10%.

17. ASSAY SPECIFICITY

CROSS REACTIVITY –

The HO-1 EIA detects Human HO-1 and does not cross react with Human HO 2 or HO 3, the other known heme oxygenase isoforms. This kit is not recommended for measuring rat or mouse HO-1.

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed

19. NOTES

UK, EU and ROW
Email: technical@abcam.com | Tel: +44-(0)1223-696000

Austria
Email: wissenschaftlicherdienst@abcam.com | Tel: 019-288-259

France
Email: supportscientifique@abcam.com | Tel: 01-46-94-62-96

Germany
Email: wissenschaftlicherdienst@abcam.com | Tel: 030-896-779-154

Spain
Email: soportecientifico@abcam.com | Tel: 911-146-554

Switzerland
Email: technical@abcam.com
Tel (Deutsch): 0435-016-424 | Tel (Français): 0615-000-530

US and Latin America
Email: us.technical@abcam.com | Tel: 888-77-ABCAM (22226)

Canada
Email: ca.technical@abcam.com | Tel: 877-749-8807

China and Asia Pacific
Email: hk.technical@abcam.com | Tel: 400 921 0189 / +86 21 2070 0500

Japan
Email: technical@abcam.co.jp | Tel: +81-(0)3-6231-0940
www.abcam.com | www.abcam.cn | www.abcam.co.jp