HMOX1 ELISA Kit Human (OKBB00836)

Lot# KF0007

Instructions for Use

For the quantitative measurement of HMOX1 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

Variation between lots can occur. Refer to the manual provided with the kit.

This product is intended for research use only

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1. Background

Principle

Aviva's human HO-1 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for HO-1 has been precoated onto 96-well plates. Standards(E.coli, M1-M288) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for HO-1 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human HO-1 amount of sample captured in plate.

Target Background

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.

General Specifications

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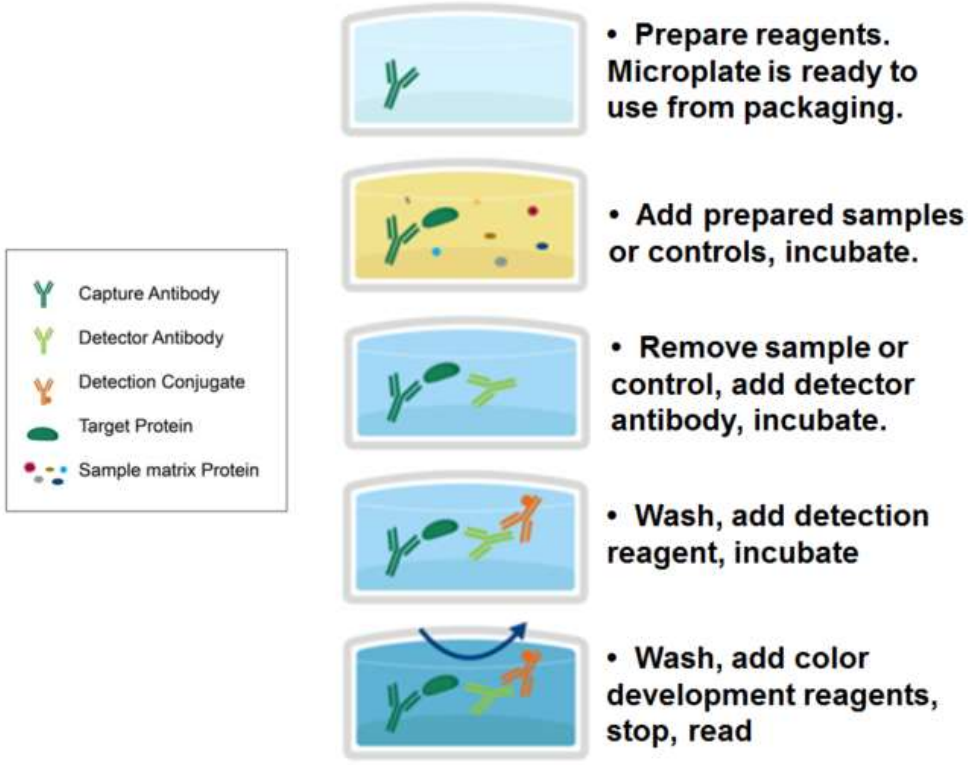
Range	93.7 – 6,000 pg/mL
LOD	< 10 pg/mL (Derived by linear regression of OD_{450} of the Mean Blank + 2xSD)
Specificity	Human HMOX1 UniProt: P09601 Gene ID: 3162 Target Alias: HO-1, HSP32, HMOX1D, bK286B10
Cross-Reactivity	No detectable cross-reactivity with other relevant proteins

2. Assay Summary



3. Storage and Stability

- Upon receipt store kit at -20°C until expiration date. Avoid multiple freeze/thaw cycles.

4. Kit Components

- The following reagents are the provided contents of the kit.

Description	Quantity	Storage Conditions
96-Well Plate Pre-coated with Anti-Human HMOX1 Antibody	96 Wells (12 x 8 Well Strips)	Store at -20°C until expiration date
Lyophilized Recombinant Human HMOX1 Standard	2 x 10 ng	
100X Biotinylated Anti- Human HMOX1 Antibody	1 x 130 µL	
100X Avidin-Biotin-Peroxidase Complex (ABC)	1 x 130 µL	
Sample Diluent Buffer	1 x 30 mL	
Antibody Diluent Buffer	1 x 12 mL	
ABC Diluent Buffer	1 x 12 mL	
TMB Color Developing Agent	1 x 10 mL	
TMB Stop Solution	1 x 10 mL	
10X Wash Buffer	1 x 30 mL	

5. Precautions

- Read instructions fully prior to beginning use of the assay kit.
- Any deviations or modifications from the described method or use of other reagents could result in a reduction of performance.
- Reduce exposure to potentially harmful substances by wearing personal protective lab equipment including lab coats, gloves and glasses.
- For information on hazardous substances included in the kit please refer to the Material Safety Data Sheet (MSDS).
- Kit cannot be used beyond the expiration date on the label.

6. Required Materials Not Supplied

- Microplate reader capable of reading absorbance at 450 nm.
- Automated plate washer (optional).
- Pipettes capable of precisely dispensing 0.5 µL through 1 mL volumes of aqueous solutions.
- Pipettes or volumetric glassware capable of precisely measuring 1 mL through 100 mL of aqueous solutions.
- New, clean tubes and/or micro-centrifuge tubes for the preparation of standards or samples.
- Absorbent paper or paper toweling.
- Distilled or deionized ultrapure water.

7. Technical Application Tips

- Do not mix or substitute components from other kits.
- To ensure the validity of experimental operation, it is recommended that pilot experiments using standards and a small selection of sample dilutions to ensure optimal dilution range for quantitation.
- Samples exhibiting OD measurements higher than the highest standard should be diluted further in the appropriate sample dilution buffers.
- Prior to using the kit, briefly spin component tubes to collect all reagents at the bottom.
- Replicate wells are recommended for standards and samples.
- Cover microplate while incubating to prevent evaporation.
- Do not allow the microplate wells dry at any point during the assay procedure.
- Do not reuse tips or tube to prevent cross contamination.
- Avoid causing bubbles or foaming when pipetting, mixing or reconstituting.
- Completely remove of all liquids when washing to prevent cross contamination.
- Prepare reagents immediately prior to use and do not store, with the exception of the top standard.
- Equilibrate all materials to ambient room temperature prior to use (standards exception).
- For optimal results for inter- and intra-assay consistency, equilibrate all materials to room temperature prior to performing assay (standards exception).
- Pipetting less than 1 µL is not recommended for optimal assay accuracy.
- Once the procedure has been started, all steps should be completed without interruption. Ensure that all reagents, materials and devices are ready at the appropriate time.
- Incubation times will affect results. All wells should be handled in the same sequential order and time intervals for optimal results.
- Samples containing bilirubin, precipitates or fibrin strands or are hemolytic or lipemic might cause inaccurate results due to interfering factors.
- TMB Substrate is easily contaminated and should be colorless or light blue until added to plate. Handle carefully and protect from light.

8. Reagent Preparation

- Equilibrate all materials to room temperature prior to use and use immediately.

8.1. HMOX1 Assay Standards

8.1.1. Prepare the **HMOX1** standards no greater than 2 hours prior to performing experiment. Standards should be held on ice until use in the experiment.

8.1.2. Reconstitute one of the provided 10 ng **Lyophilized Recombinant Human HMOX1 Standard**. Use one for each experiment. Prepare a stock 10,000 pg/mL **Human HMOX1 Standard** by reconstituting one tube of **Lyophilized Recombinant Human HMOX1 Standard** as follows:

8.1.2.1. Gently spin or tap the vial to collect all material at the bottom.

8.1.2.2. Add 1 mL of **Sample Diluent Buffer** to the vial.

8.1.2.3. Seal then mix gently and thoroughly.

8.1.2.4. Leave the vial to sit at ambient temperature for 10 minutes.

8.1.3. Prepare a set of seven serially diluted standards as follows:

8.1.3.1. Label tubes with numbers 1 – 8.

8.1.3.2. Add 300 µL of **Sample Diluent Buffer** to Tube #'s 2 – 8.

8.1.3.3. Prepare a **6,000 pg/mL Standard #1** by adding 600 µL of the 10,000 pg/mL reconstituted **Human HMOX1 Standard** to 400 µL of **Sample Diluent Buffer** in Tube#1. Mix gently and thoroughly.

8.1.3.4. Prepare **Standard #2** by adding 300 µL of **6,000 pg/mL Standard #1** from Tube #1 to Tube #2. Mix gently and thoroughly.

8.1.3.5. Prepare **Standard #3** by adding 300 µL of **Standard #2** from Tube #2 to Tube #3. Mix gently and thoroughly.

8.1.3.6. Prepare further serial dilutions through Tube #7. Reference the table below as a guide for serial dilution scheme.

8.1.3.7. Tube #8 is a blank standard (only **Sample Diluent Buffer**), which should be included with every experiment.

Standard Number (Tube)	Standard To Dilute	Volume Standard to Dilute (µL)	Volume Sample Diluent Buffer (µL)	Total Volume (µL)	Final Concentration
1	10,000 pg/mL Reconstituted HMOX1 Standard	600	400	1,000	6,000 pg/mL
2	6,000 pg/mL	300	300	600	3,000 pg/mL
3	3,000 pg/mL	300	300	600	1,500 pg/mL
4	1,500 pg/mL	300	300	600	750 pg/mL
5	750 pg/mL	300	300	600	375 pg/mL
6	375 pg/mL	300	300	600	187.5 pg/mL
7	187.5 pg/mL	300	300	600	93.7 pg/mL
8	NA	0	300	300	0.0 (Blank)

8.2. 1X Biotinylated Anti-Human HMOX1 Antibody

8.2.1. Prepare the **1X Biotinylated Human HMOX1 Antibody** immediately prior to use by diluting the **100X Biotinylated Human HMOX1 Antibody** 1:00 with **Antibody Diluent Buffer**.

8.2.2. For each well strip to be used in the experiment prepare 100 µL by adding 1 µL of **100X Biotinylated Human HMOX1 Antibody** to 99 µL **Antibody Diluent Buffer**.

8.2.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.3. 1X Avidin-Biotin-Peroxidase Complex (ABC)

8.3.1. Prepare the **1X Avidin-Biotin-Peroxidase Complex (ABC)** immediately prior to use by diluting the **100X Avidin-Biotin-Peroxidase Complex (ABC) 1:100** with **ABC Dilution Buffer**.

8.3.2. For each well to be used in the experiment prepare 100 µL, by adding 1 µL of **100X Avidin-Biotin-Peroxidase Complex (ABC)** to 99 µL ABC Dilution Buffer.

8.3.3. Mix thoroughly and gently. Hold no longer than 2 hours prior to using in procedure. Do not store at 1X concentration for future use.

8.4. 1X Wash Buffer

8.4.1. Add 270 mL of ultra-pure water to a clean > 500 mL bottle or other vessel.

8.4.2. Add the entire 30 mL contents of the **10X Wash Buffer** bottle to the water.

8.4.3. Seal and mix gently by inversion. Avoid foaming or bubbles.

8.4.4. Store the **1X Wash Buffer** at room temperature until ready to use in the procedure. Store the prepared **1X Wash Buffer** at 4°C for no longer than 1 week. Do not freeze.

8.5. Microplate Preparation

- Micro-plates are provided ready to use and do not require rinsing or blocking.
- Unused well strips should be returned to the original packaging, sealed and stored at 4°C.
- Equilibrate microplates to ambient temperatures prior to opening to reduce potential condensation.

9. Sample Preparation Guidelines

9.1. Sample Preparation and Storage

- Samples must be tested to determine if the kit is valid.
- Store samples to be assayed at 4°C for 24 hours prior being assayed.
- For long term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

General Sample Preparation Guidelines:

- **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°C.
- **Plasma** - Collect plasma using EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1,000 x g at 4°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.
- **Other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze/thaw cycles.

9.2. Sample Dilution

Target protein concentration must be estimated and appropriate sample dilution selected such that the final target protein concentration falls near the middle of the assay linear dynamic range. Samples exhibiting saturation should be further diluted.

- Prior to performing the full experiment, test a serially diluted representative sample.
  - Alternately, a small pool of several samples can also be used with this same method if sample volume is limited.

  Or
  - Refer to published literature for expected concentrations and derive the optimal dilution level based on the expected dynamic range of the kit
- Dilute samples using **Sample Diluent Buffer**.
- Mix diluted samples gently and thoroughly.
- Pipetting less than 2 µL is not recommended for optimal assay accuracy.

10. Assay Procedure

• The ABC Working Solution, TMB Color Developing Agent and TMB Stop Solution must be kept warm at 37°C for 30 minutes prior to use. Equilibrate all other reagents and materials to ambient room temperature prior to use in the procedure.

• To control for small potential variations in micro well-plate and day to day ambient temperature fluctuations, equilibrate all reagents prior to use and perform all incubation steps at to 37°C for optimal consistency and reproducibility.

10.1. Add 100 µL of serially titrated standards, diluted samples or blank into wells of the pre-coated well plate. At least two replicates of each standard, sample or blank is recommended.

10.2. Cover the plate with the plate sealer and incubate at 37°C for 90 minutes.

10.3. Remove the plate sealer and discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.4. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.5. Add 100 µL of prepared **1X Biotinylated Anti-Human HMOX1 Antibody to each well.**

10.6. Cover with the plate sealer and incubate at 37°C for 60 minutes.

10.7. Wash plate 3 times with **1X Wash Buffer as** follows:

10.7.1. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.7.2. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.7.3. Add 300 µL of **1X Wash Buffer** to each assay well.

10.7.4. Incubate for 1 minute.

10.7.5. Repeat steps 10.7.1 through 10.7.4 **two** more times.

10.8. Add 100 µL of prepared **1X Avidin-Biotin-Peroxidase Complex (ABC) into** each well, cover with plate sealer and incubate at 37°C for 30 minutes.

10.9. Wash plate 5 times with **1X Wash Buffer as** follows:

10.9.1. Discard the liquid in the wells by rigorously flicking into an acceptable waste receptacle.

10.9.2. Gently blot any remaining liquid from the wells by tapping inverted on the benchtop onto paper toweling. Do not allow the wells to completely dry at any time.

10.9.3. Add 300 µL of 1X Wash Buffer to each assay well.

10.9.4. Incubate for 1 minute.

10.9.5. Repeat steps 10.9.1 through 10.9.4 **four** more times.

10.10. Add 90 µL of **TMB Color Developing Agent** to each well, cover with plate sealer and incubate at 37°C **in the dark** for 15-25 minutes.

(NOTE: optimal incubation time must be determined by the user. Optimal development can be visualized by blue shading in the top four standard wells, while the remaining standards are still clear.)

10.11. Add 100 µL of **TMB Stop Solution** to each well. Well color should change to yellow immediately.

10.12. Read the O.D. absorbance at 450 nm with a standard microplate reader within 30 minutes of stopping the reaction in step 10.11.

11. Calculation of Results

For analysis of the assay results, calculate the **Relative OD_{450}** for each test or standard well as follows:

(Relative OD_{450}) = (Well OD_{450}) – (Mean Blank Well OD_{450})

The standard curve is generated by plotting the mean replicate **Relative OD_{450}** of each standard serial dilution point vs. the respective standard concentration. The **HMOX1** concentration contained in the samples can be interpolated by using linear regression of each mean sample **Relative OD_{450}** against the standard curve. This is best achieved using curve fitting software. A standard curve should be generated each time the test is performed.

Note: if wavelength correction readings were available, subtract the readings at 540 nm or 570 nm from the readings at 450 nm. This may provide greater reading accuracy.

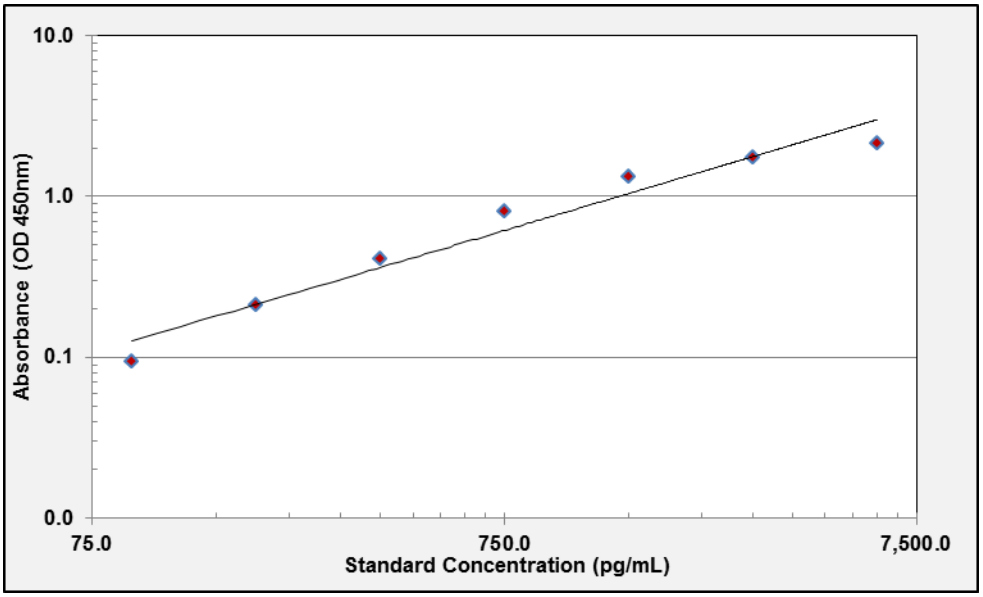
Note: if the samples measured were diluted, multiply the derived mean sample concentration by the dilution factor for a final sample concentration.

12. Typical Expected Data

12.1. Typical absorbance values. Expected absorbance for standards when TMB incubation is performed for 20 minutes at 37°C and measured at OD_{450}.

Standard Number	8	7	6	5	4	3	2	1
Concentration (pg/ml)	0	93.7	187.5	375	750	1500	3000	6000
OD_{450}	0.010	0.095	0.210	0.407	0.814	1.333	1.763	2.133

12.2. Typical standard curve. This standard curve is for demonstration purposes only. An assay specific standard curve should be performed with each assay.



12.3. Reproducibility

	Intra-Assay			Inter-Assay		
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	5.0	4.8	9.2	6.5	5.9

13. Technical Resources

13.1 Technical Support:

For optimal service please be prepared to supply the lot number of the kit used.

USA

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