



AssayMax Human Hemopexin ELISA Kit (Plasma and Serum Samples)

Catalog No. EH1001-1

Introduction

Hemopexin is a heme-binding plasma glycoprotein which, after haptoglobin, forms the second line of defense against hemoglobin-mediated oxidative damage during intravascular hemolysis. A decrease in plasma hemopexin concentration reflects a recent release of heme compounds in the extracellular compartment. Heme-hemopexin complexes are delivered to hepatocytes by receptor-mediated endocytosis after which hemopexin is recycled to the circulation (1). Studies indicated that increased hemopexin level associate with minimal change disease (2), sporadic Alzheimer's disease (3), heavy-drinking chronic alcoholics (4), hemolytic anemias, chronic neuromuscular diseases and acute intermittent porphyria (5).

Principal of the Assay

The AssayMax Human Hemopexin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human hemopexin in plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures human hemopexin in less than 2 hours. A polyclonal antibody specific for human hemopexin has been pre-coated onto a 96-well microplate with removable strips. Hemopexin in standards and samples is competed by a biotinylated hemopexin sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standards, biotinylated-protein, and SP conjugate) as instructed, prior to running the assay.**
- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acid solution

Reagents

- **Hemopexin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human hemopexin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Hemopexin Standard:** Human Hemopexin in a buffered protein base (3.5 μg , lyophilized).
- **Biotinylated Hemopexin:** 1 vial, lyophilized.
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x):** A 20-fold concentrated buffered surfactant (30 ml).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 μl).
- **Chromogen Substrate:** A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution:** A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Store components of the kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Store SP Conjugate at -20⁰C
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8⁰C
- Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.
- Diluent (1x) may be stored for up to 1 month at 2-8⁰C.
- Store Standard and Biotinylated Protein at 2-8⁰C before reconstituting with Diluent and at -20⁰C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 μl , 20-200 μl , 200-1000 μl and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute samples 1:400 into MIX Diluent. Store samples at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:400 into MIX Diluent. Store serum at -20⁰C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.

- **Standard Curve:** Reconstitute the 3.5 µg of Hemopexin Standard with 0.7 ml of MIX Diluent to generate a solution of 5 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (5 µg/ml) 1:4 with MIX Diluent to produce 1.25, 0.313 and 0.078 µg/ml solutions. MIX Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20°C.

Standard Point	Dilution	[Hemopexin] (µg/ml)
P1	Standard (5 µg/ml)	5.000
P2	1 part P1 + 3 parts MIX Diluent	1.250
P3	1 part P2 + 3 parts MIX Diluent	0.313
P4	1 part P3 + 3 parts MIX Diluent	0.078
P5	MIX Diluent	0.000

- **Biotinylated Hemopexin (8x):** Dilute Biotinylated Hemopexin with 4 ml MIX Diluent to produce to stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:8 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute the Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-30°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of standard or sample per well, and immediately add 25 µl of Biotinylated Hemopexin to each well (on top of the Standard or sample) and mix gently. Cover wells with a sealing tape and incubate for one hour. Start the timer after the last sample addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µl of Wash Buffer and then invert the plate, decant the contents; hit it 4-5 times on absorbent paper towel to completely remove the liquid.
- Add 50 µl of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 µl of Chromogen Substrate per well and incubate for about 10 minutes or till the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 µl of Stop Solution to each well. The color will change from blue to yellow.
- Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some

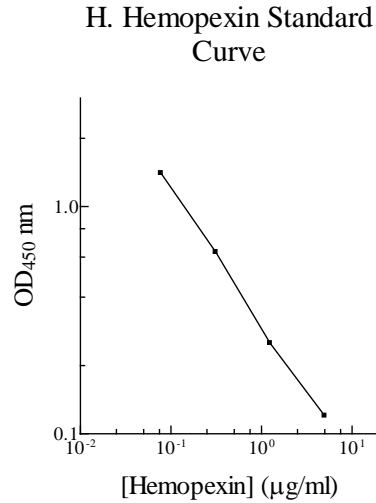
unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable dose of Hemopexin is typically 0.07 µg/ml.
- Intra-assay and inter-assay coefficients of variation were 4.9% and 7.3% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:200	104%	105%
1:400	100%	101%
1:800	96%	95%

Recovery

Standard Added Value	0.2 – 2 ug/ml
Recovery %	82-114 %
Average Recovery %	98 %

Cross-Reactivity

Species	% Cross Reactivity
Canine	< 2
Monkey	> 40 (Suggest dilution 1:100 for plasma/serum)
Mouse	< 3
Rat	< 2
Swine	< 4

References

1. Delanghe JR et al. (2001) Clin Chim Acta. 312(1-2): 13-23
2. Bakker WW et al. (2005) Pediatr Nephrol. 20(10): 1410-5
3. Yu HL et al. (2003) Proteomics 3(11): 2240-8
4. Kristensson-Aas A et al. (1986) Eur J Clin Invest. 16(2): 178-83
5. Suzuki K et al. (2004) Nippon Rinsho. 62(3): 577-86

Version 2.6

Related Products

- EH2001-1 AssayMax Human Hemopexin ELISA Kit (Urine, Saliva, Milk and Cell Culture Supernatants samples)