



Human Hemopexin ELISA Kit

Vertrieb:

L O X O GmbH Immunbiologie Biochemie, Produkte und Systeme
Postfach 11 30 69215 Dossenheim
Telefon +49 (0) 62 21 - 86 80 23 **FAX** +49 (0) 62 21 - 86 80 255
E-Mail: info@loxo.de **Internet:** www.loxo.de

Assaypro LLC
30 Triad South Drive
St. Charles, MO 63304
T (636) 447-9175
F (636) 447-9475
www.assaypro.com

Hinweis/Note:

Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Symbol Key

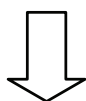


Consult instructions for use.

Assay Summary

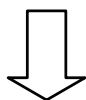
Add 25 μ l of Standard/ Sample
and 25 μ l of Biotinylated Protein per well.

Incubate 1 hour.



Wash, then add 50 μ l of
SP Conjugate per well.

Incubate 30 minutes.



Wash, then add 50 μ l of
Chromogen Substrate per well.

Incubate 12 minutes.



Add 50 μ l of Stop Solution per well.

Read at 450 nm immediately.

AssayMax Human Hemopexin ELISA Kit

Catalog No. EH1001-1
Sample Insert/Reference Only

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 levels associate with minimal change disease (MCD) (2), sporadic Alzheimer's disease (AD) (3), chronic alcoholism (4), hemolytic anemias, chronic neuromuscular diseases, and acute intermittent porphyria (5).

Principle of the Assay

The AssayMax Human Hemopexin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human hemopexin in plasma and serum samples. 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 acidic solution.

Reagents

- **Human 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 precut, pressure sensitive sealing tapes, which can be cut to fit the format of the individual assay.
- **Human Hemopexin Standard:** Human hemopexin in a buffered protein base (17.5 µg, lyophilized).
- **Biotinylated Human 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

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard and Biotinylated Protein at 2-8°C before reconstituting with diluent and at -20°C 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, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes. Dilute samples 1:400 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes, and remove serum. Dilute samples 1:400 into MIX Diluent and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. 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 Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 17.5 µg of Human Hemopexin Standard with 3.5 ml of MIX Diluent to generate a 5 µg/ml standard solution. 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 and used within 30 days.

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 Human Hemopexin (8x):** Reconstitute Biotinylated Human Hemopexin with 4 ml MIX Diluent to produce a 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 and used within 30 days.

- **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, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 25 µl of Human Hemopexin Standard or sample per well, and immediately add 25 µl of Biotinylated Human Hemopexin to each well (on top of the standard or sample) and mix gently. Cover wells with a sealing tape and incubate for 1 hour. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material 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 12 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.

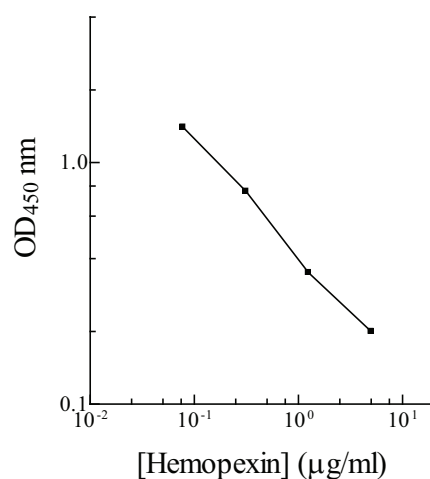
Data Analysis

- 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.

H. Hemopexin Standard Curve



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 7.4% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:200	103%	105%
1:400	99%	100%
1:800	94%	95%

Recovery

Standard Added Value	0.2 – 2 µg/ml
Recovery %	82 – 117%
Average Recovery %	98%

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Monkey	20%
Mouse	None
Rat	None
Swine	None
Bovine	None
Rabbit	None
Human	100%

Reference Value

- Normal human hemopexin plasma levels range from 0.2 to 1 mg/ml.

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 3.3

Related Products

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