



AssayMax Human Haptoglobin ELISA Kit (Plasma and Serum)

Catalog Number EH1003-1

Introduction

Haptoglobin (Hpt) is a plasma protein with hemoglobin-binding capacity, and plasma glycoproteins that form a stable complex with hemoglobin to aid the recycling of heme iron. It is a well-known marker of hemolysis (1). High Haptoglobin level in plasma was associated with an increased cardiovascular risk in obese men (2), inflammation (3), atherosclerosis (4), and systemic sclerosis (5).

Principal of the Assay

The AssayMax Human Haptoglobin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human haptoglobin in plasma and serum. This assay employs a quantitative competitive enzyme immunoassay technique that measures human haptoglobin in less than 2 hours. A polyclonal antibody specific for human Haptoglobin has been pre-coated onto a 96-well microplate with removable strips. Haptoglobin in standards and samples is competed by a biotinylated Haptoglobin 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

- 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

- **Human Haptoglobin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against human Haptoglobin.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Human Haptoglobin Standard:** Human Haptoglobin in a buffered protein base (20 µg, lyophilized).
- **Biotinylated Haptoglobin:** 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 (90 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 kit at 2-8⁰C or -20⁰C upon arrival up to the expiration date.
- Opened MIX Diluent may be stored for up to 1 month at 2-8⁰C. Store reconstituted reagents at -20⁰C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

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 2000 x g for 10 minutes and assay. Dilute samples 1:2000 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 1:2000 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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **MIX Diluent Concentrate (10x):** Dilute the MIX Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8⁰C.
- **Standard Curve:** Reconstitute the 20 µg of Haptoglobin Standard with 1 ml of MIX Diluent to generate a solution of 20 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (20 µg/ml) 1:4 with MIX Diluent to produce 5, 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 -20⁰C.

Standard Point	Dilution	[Haptoglobulin] ($\mu\text{g/ml}$)
P1	Standard (20 $\mu\text{g/ml}$)	20.000
P2	1 part P1 + 3 parts MIX Diluent	5.000
P3	1 part P2 + 3 parts MIX Diluent	1.250
P4	1 part P3 + 3 parts MIX Diluent	0.313
P5	1 part P4 + 3 parts MIX Diluent	0.078
P6	MIX Diluent	0.000

- **Biotinylated Haptoglobulin (2X):** Dilute Biotinylated Haptoglobulin with 4 ml MIX Diluent to produce a two-fold stock solution. Allow to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 with the MIX Diluent. Any remaining solution should be frozen at $< -20^{\circ}\text{C}$.
- **Wash Buffer Concentrate (20x):** 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

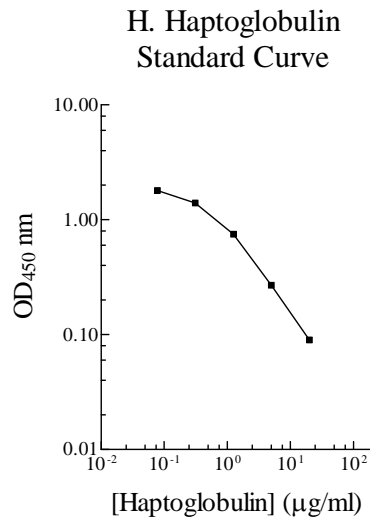
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature ($20-30^{\circ}\text{C}$).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant 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 Haptoglobulin 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. Invert the plate to decant the contents, and hit it 4-5 times on absorbent paper towel to completely remove liquid at each step.
- 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 five times with 200 μl of Wash Buffer.
- 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**. Please note that after the reaction is stopped for about 10 minutes, some black particles may be generated at high concentration point, which will reduce the readings.

Data Analysis

- Calculate the mean value of the 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 Haptoglobulin is typically 20 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.1% and 8.0% respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value	
	Plasma	Serum
1:2000	100%	97%
1:4000	100%	101%
1:8000	107%	106%

Recovery

Standard Added Value	0.1 – 5 ug/ml
Recovery %	82-119 %
Average Recovery %	100.5 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	< 0.05
Monkey	< 5 (suggest dilution 1:20 for plasma)
Mouse	< 10 (suggest dilution 1:100 for plasma)
Rat	< 0.2
Swine	< 0.05

References

- (1) Van Vlierberghe H *et al.* (2004) *Clin Chim Acta.* 345(1-2): 35-42
- (2) Engstrom G *et al.* (2004) *Arterioscler Thromb Vasc Biol.* 24(8): 1498-502
- (3) Rocha-Pereira P *et al.* (2004) *Br J Dermatol.* 150(5): 917-28
- (4) Matuszek MA *et al.* (2003) *Atherosclerosis* 168(2): 389-96
- (5) Kucharz EJ *et al.* (2000) *Clin Rheumatol* 19(2): 165-6

Version 4.0

Related Products

- EH2003-1 AssayMax Human Haptoglobin ELISA Kit (Urine & Cell Culture Samples)