AssayMax Human Haptoglobin ELISA Kit

Catalog Number EH1003-1
Lot #

Introduction

Haptoglobin (Hpt) is a plasma protein with hemoglobin-binding capacity, and a 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 Haptoglobulin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of human haptoglobulin in plasma, serum and urine. This assay employs a quantitative competitive sandwich enzyme immunoassay technique that measures human haptoglobulin 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 (100 µg, lyophilized).

- **Biotinylated Haptoglobin:** 1 vial, lyophilized.
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (120 µl).
- **EIA Diluent Concentrate (10x)**: A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (10x)**: A 10-fold concentrated buffered surfactant (2 x 30 ml).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydroxychloric acid (12 ml) to stop the chromogen substrate reaction.

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and Biotinylated Haptoglobin at -20^{0}C 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, 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 2,000x g for 10 minutes and assay. Dilute samples 1:4000 into EIA Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes. Remove serum and assay. Dilute samples 1:4000 into EIA Diluent. Store serum at -20^{0}C or below. Avoid repeated freeze-thaw cycles
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute samples 1:2 into EIA Diluent. Store samples at at -20^{0}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. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.
- **Standard Curve:** Reconstitute the 100 µg of Haptoglobin Standard with 2 ml of EIA Diluent to generate a solution of 50 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 (50 µg/ml) 1:4 with EIA Diluent to produce 12.5, 3.13, 0.78, 0.195, and 0.049 µg/ml solutions. EIA Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at < -20^{0}C.

Standard Point	Dilution	[Haptoglobin] (μg/ml)
P1	Standard (50 μg/ml)	50.00
P2	1 part P1 + 3 parts EIA Diluent	12.5
P3	1 part P2 + 3 parts EIA Diluent	3.13
P4	1 part P3 + 3 parts EIA Diluent	0.78
P5	1 part P4 + 3 parts EIA Diluent	0.195
P6	1 part P5 + 3 parts EIA Diluent	0.049
P8	EIA Diluent	0.000

- **Biotinylated Haptoglobin:** Dilute Biotinylated Haptoglobin with 4 ml EIA Diluent to produce a working solution. Any remaining solution should be frozen at < -20^{0}C.
- **EIA Diluent Concentrate (10x):** Dilute the EIA Diluent 1:10 with reagent grade water.
- **Wash Buffer Concentrate (10x):** Dilute the Wash Buffer Concentrate 1:10 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent.

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^{0}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 Haptoglobin 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 sample addition.
- Wash five times with 200 μl of Wash Buffer. Invert the plate and decant the contents, and hit it 4-5 times on absorbent paper towel to complete 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 8 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.

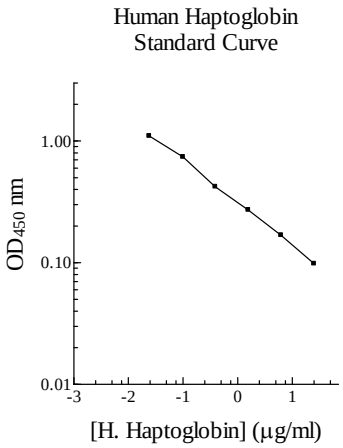
Data Analysis

- 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 semi-log curve fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the plasma value by the dilution factor of 8000, serum value by the dilution factor of 4000, and urine value by the dilution factor of 2.

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 Haptoglobin is typically 20 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.9% and 9.4% respectively.
- No significant cross-reactivity or interference was observed.

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

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