



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

Catalog Number EH2003-1

Introduction

Haptoglobin (Hpt) is a plasma protein with hemoglobin-binding capacity, and a plasma glycoprotein that forms 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 kit is designed for detection of human urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures haptoglobin in 4 hours. A polyclonal antibody specific for haptoglobin has been pre-coated onto a microplate. Haptoglobin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for haptoglobin, which is recognized by a 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 (400 ng, lyophilized).
- **Biotinylated Haptoglobin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against human Haptoglobin (80 µl).
- **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

- **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20⁰C 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 1:4 into MIX Diluent. Store samples at -20⁰C 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.
- **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 400 ng of Haptoglobin Standard with 1 ml of MIX Diluent to generate a solution of 400 ng/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 (400 ng/ml) 1:4 with MIX Diluent to produce 100, 25, 6.25, and 1.56 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at < -20⁰C.

Standard Point	Dilution	[Haptoglobin] (ng/ml)
P1	Standard (400 ng/ml)	400.00
P2	1 part P1 + 3 parts MIX Diluent	100.00
P3	1 part P2 + 3 parts MIX Diluent	25.00
P4	1 part P3 + 3 parts MIX Diluent	6.25
P5	1 part P4 + 3 parts MIX Diluent	1.56
P6	MIX Diluent	0.00

- **Biotinylated Haptoglobin Antibody (100x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°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

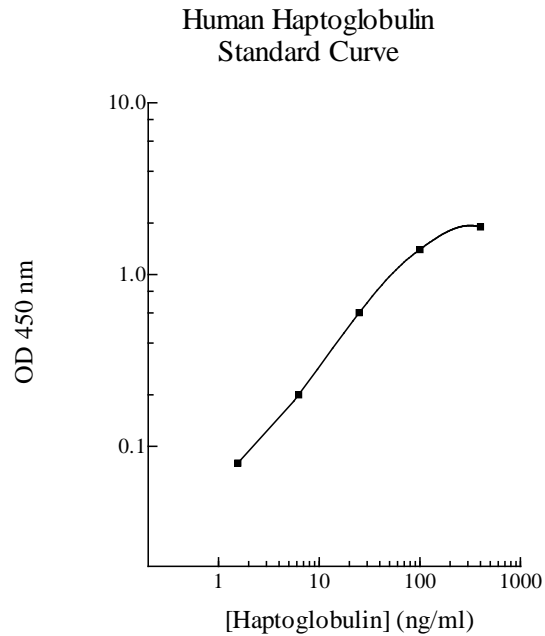
- Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature ($20\text{-}30^{\circ}\text{C}$).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add $50\ \mu\text{l}$ of Standard or sample per well. Cover wells and incubate for two hours. Start the timer after the last sample addition.
- Wash five times with $200\ \mu\text{l}$ 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 $50\ \mu\text{l}$ of Biotinylated Haptoglobin Antibody to each well and incubate for one hour.
- Wash five times with $200\ \mu\text{l}$ of Wash Buffer as above.
- Add $50\ \mu\text{l}$ of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash five times with $200\ \mu\text{l}$ of Wash Buffer as above.
- Add $50\ \mu\text{l}$ of Chromogen Substrate per well and incubate for about 8 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add $50\ \mu\text{l}$ 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 $450\ \text{nm}$ **immediately**. 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 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 $450\ \text{nm}$ 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 Haptoglobin is typically 0.3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 5.0% and 7.1 % respectively.

Linearity

Sample Dilution	Average Percentage of Expected Value
	Urine
1:2	100%
1:4	102%
1:8	101%
1:16	100%

Recovery

Standard Added Value	2 – 200 ng/ml
Recovery %	83-119 %
Average Recovery %	101 %

Cross-Reactivity

Species	% Cross Reactivity
Beagle	None
Bovine	None
Monkey	< 10
Mouse	< 2
Rat	< 0.1
Swine	None

- 10% FBS in culture media will not affect the assay.

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 2.2

Related Products

- EH1003-1 AssayMax Human Haptoglobin ELISA Kit (Plasma & Serum Samples)