HP (Bovine) ELISA Kit

Catalog Number KA1850

96 assays

Version: 01

Intended for research use only

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Introduction and Background

A. Overview

Haptoglobulin (HP; 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 haptoglobulin level in plasma was associated with an increased cardiovascular risk in obese men (2), inflammation (3), atherosclerosis (4), and systemic sclerosis (5).

B. Test Principle

The HP (Bovine) ELISA Kit is designed for detection of Haptoglobulin in bovine urine and cell culture supernatants. This assay employs a quantitative sandwich enzyme immunoassay technique that measures haptoglobulin in less than 4 hours. A polyclonal antibody specific for haptoglobulin has been pre-coated onto a microplate. Haptoglobulin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for haptoglobulin, 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.

C. Notice for Application of Kit

- ✓ **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.

Material and Method

A. List of component

- ✓ **Bovine Haptoglobulin Microplate:** A 96-well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against bovine Haptoglobulin.
- ✓ **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- ✓ **Bovine Haptoglobulin Standard:** Bovine Haptoglobulin in a buffered protein base (480 ng, lyophilized).
- ✓ **Biotinylated Haptoglobulin Antibody (100x):** A 100-fold concentrated biotinylated polyclonal antibody against bovine Haptoglobulin (80 µL).
- ✓ **EIA Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 mL).
- ✓ Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 mL, 2 bottles).
- ✓ 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).

B. Additional Required Materials But Not Provided

- ✓ 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.

C. Sample Collection, Preparation and Storage

- ✓ **Cell Culture Supernatants:** Centrifuge cell culture media at 2000 xg for 10 minutes to remove debris. Collect supernatants and assay. Store the remaining samples at -20℃ or below. Avoid repeated freeze-thaw cycles.
- ✓ **Urine:** Collect urine using sample pot. Centrifuge samples at 800 xg for 10 minutes and assay. Dilute samples 1:2 into EIA Diluent. Store samples at -20℃ or below for up to 3 months. Avoid repeated freeze-thaw cycles.

D. 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.
- ✓ EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1 month at 2-8℃.
- ✓ **Standard Curve:** Reconstitute the 480 ng of Haptoglobulin Standard with 2 mL of EIA Diluent to generate a solution of 240 ng/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 (240 ng/mL) 1:2 with EIA Diluent to produce 120, 60, 30, 15, 7.5, and 3.75 ng/mL solutions. EIA Diluent

serves as the zero standard (0 ng/mL). Any remaining solution should be frozen at < -20℃.

Standard Point	Dilution	[B. Haptoglobulin] (ng/mL)
P1	Standard (240 ng/mL)	240.00
P2	1 part P1 + 1 part EIA Diluent	120.00
P3	1 part P2 + 1 part EIA Diluent	60.00
P4	1 part P3 + 1 part EIA Diluent	30.00
P5	1 part P4 + 1 part EIA Diluent	15.00
P6	1 part P5 + 1 part EIA Diluent	7.50
P7	1 part P6 + 1 part EIA Diluent	3.75
P8	EIA Diluent	0.00

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

E. Stability and storage

- ✓ Store components of the kit at 2-8℃ or -20℃ upon arrival up to the expiration date.
- ✓ Store SP Conjugate and Biotinylated Antibody at -20℃.
- ✓ Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8℃.
- ✓ 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 2-8℃.
- ✓ Store Standard at 2-8℃ before reconstituting with Diluent and at -20℃ after reconstituting with Diluent.

F. Protocol

- ✓ 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℃).
- ✓ 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 µL 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 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 **Biotinylated Haptoglobulin Antibody** to each well and incubate for one hour.
- ✓ Wash the microplate as described above.
- ✓ Add 50 µL of **Streptavidin-Peroxidase Conjugate** per 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 15 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 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.

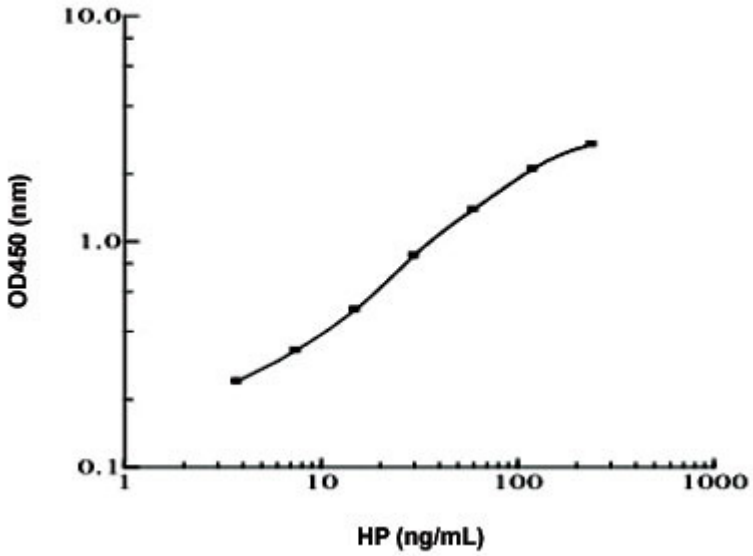
Result

A. 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.

B. 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 ~3.7 ng/mL.
- ✓ Intra-assay and inter-assay coefficients of variation were 5.1% and 7.3 % respectively.

Linearity

	Average Percentage of Expected Value
Sample Dilution	**Urine**
No Dilution	88%
1:2	96%
1:4	94%

Recovery

Standard Added Value	4 – 40 ng/mL
Recovery %	84-109 %
Average Recovery %	96 %

Cross-Reactivity

Species	**% Cross Reactivity**
Canine	25%
Bovine	100%
Monkey	10%
Mouse	5%
Rat	None
Swine	25%
Rabbit	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