Product Manual

Human Haptoglobin ELISA Kit

Catalog Number

PRB-5039 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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Creating Solutions for Life Science Research

Introduction

Haptoglobin (Hp) is a tetramer composed of two alpha and beta subunits processed and combined from a preprotein. The protein is expressed in adipose tissue, kidney, skin, lung, as well as hepatocyte cells. Haptoglobin is secreted into blood plasma where it binds free hemoglobin (Hb) and prevents Hb from reacting oxidatively. This Hb/Hp complex is removed in the spleen by the mononuclear phagocytic system. Mutations in the Hp gene (HP) or its regulatory sequences lead to ahaptoglobinemia or hypohaptoglobinemia. Mutations in the gene have also been linked to Crohn's disease, diabetic nephropathy, inflammatory disease, increased incidence of coronary artery disease in type 1 diabetes, a reduced incidence of Plasmodium falciparum malaria, primary sclerosing cholangitis, and susceptibility to idiopathic Parkinson's disease. Some studies suggest an association between certain haptoglobin phenotypes with the risk of developing schizophrenia.

Cell Biolabs' Human Haptogloblin ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of human haptoglobin in plasma, serum, urine, cell or tissue lysate samples. The kit has a detection sensitivity limit of 1 ng/mL human haptoglobin. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. PRB-5033: Human Alpha 2 Macroglobulin ELISA Kit
2. PRB-5041: Human Ceruloplasmin ELISA Kit
3. PRB-5044: Human Alpha 1 Acid Glycoprotein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Human Hp Antibody Coated Plate (Part No. 50391B): One 96-well strip plate (8 x 12).
2. Anti-Human Hp Antibody (1000X) (Part No. 50392C): One 10 µL vial.
3. Secondary Antibody, HRP Conjugate (Part No. 231704): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Human Hp Standard (Part No. 50393D): One 50 µL vial of 4 µg/mL Human Haptoglobin.

Materials Not Supplied

1. Plasma, serum, cell or tissue lysate
2. PBS containing 0.1% BSA

3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)

Storage

Upon receipt, aliquot and store the Human Hp Standard at -80ºC to avoid multiple freeze/thaw cycles. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- Anti-Human Hp Antibody and Secondary Antibody, HRP Conjugate: Immediately before use dilute Anti-Human Hp Antibody or Secondary Antibody, HRP Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Human Hp Standard

Prepare a dilution series of human Hp standards in the concentration range of 0 to 40 ng/mL into Assay Diluent (Table 1).

Standard Tubes	4 µg/mL Human Hp Standard (µL)	Assay Diluent (µL)	Human Hp (ng/mL)
1	8	792	40
2	400 of Tube #1	400	20
3	400 of Tube #2	400	10
4	400 of Tube #3	400	5
5	400 of Tube #4	400	2.5
6	400 of Tube #5	400	1.25
7	400 of Tube #6	400	0.625
8	0	400	0

Table 1. Preparation of Human Hp Standards

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Plasma: Collect blood with an anticoagulant such as heparin, citrate or EDTA and mix by inversion. Centrifuge the blood at 1000 x g at 4°C for 10 minutes. Remove the plasma and assay immediately or store samples at -80ºC for up to three months. Normal plasma samples require about 250,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. For example, for a 250,000 fold dilution prepare a serial dilution of each sample according to Table 2 below, mixing each dilution well before continuing to the next dilution step.

- Serum: Collect blood in a tube with no anticoagulant. Allow the blood to clot at room temperature for 30 minutes. Centrifuge at 2500 x g for 20 minutes. Remove the yellow serum supernatant without disturbing the white buffy layer. Assay immediately or store samples at -80ºC for up to three months. Normal serum samples require about 250,000 fold dilution with PBS containing 0.1% BSA immediately before running the ELISA. For example, for a 250,000 fold dilution prepare a serial dilution of each sample according to Table 2 below, mixing each dilution well before continuing to the next dilution step.

Sample Tubes	Plasma or Serum Sample	PBS with 0.1% BSA	Effective Dilution
1	5 µL	4995 µL	1:1000
2	5 µL of Tube #1	1245 µL	1:250,000

Table 2. Preparation of 1:250,000 dilution of plasma or serum samples.

- Urine: Harvest urine and centrifuge for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Other Biological Fluids: Centrifuge samples for 10 minutes at 1000 g at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.
- Cell or Tissue Lysate: Sonicate or homogenize sample in cold PBS and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of human Hp unknown sample or standard to the Anti-Human Hp Antibody Coated Plate. Each human Hp unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Anti-Human Hp Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.
6. Add 100 µL of the diluted Secondary Antibody, HRP Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.

8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Human Haptoglobin ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

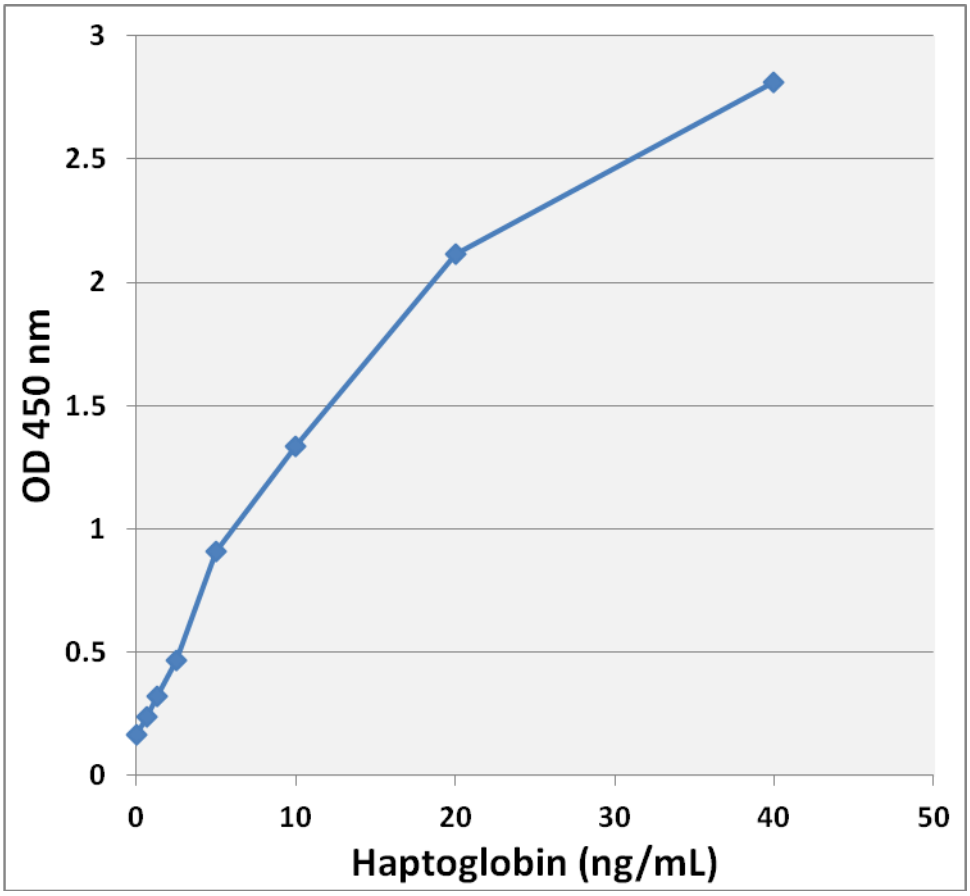

Figure 1: Human Haptoglobin ELISA Standard Curve.

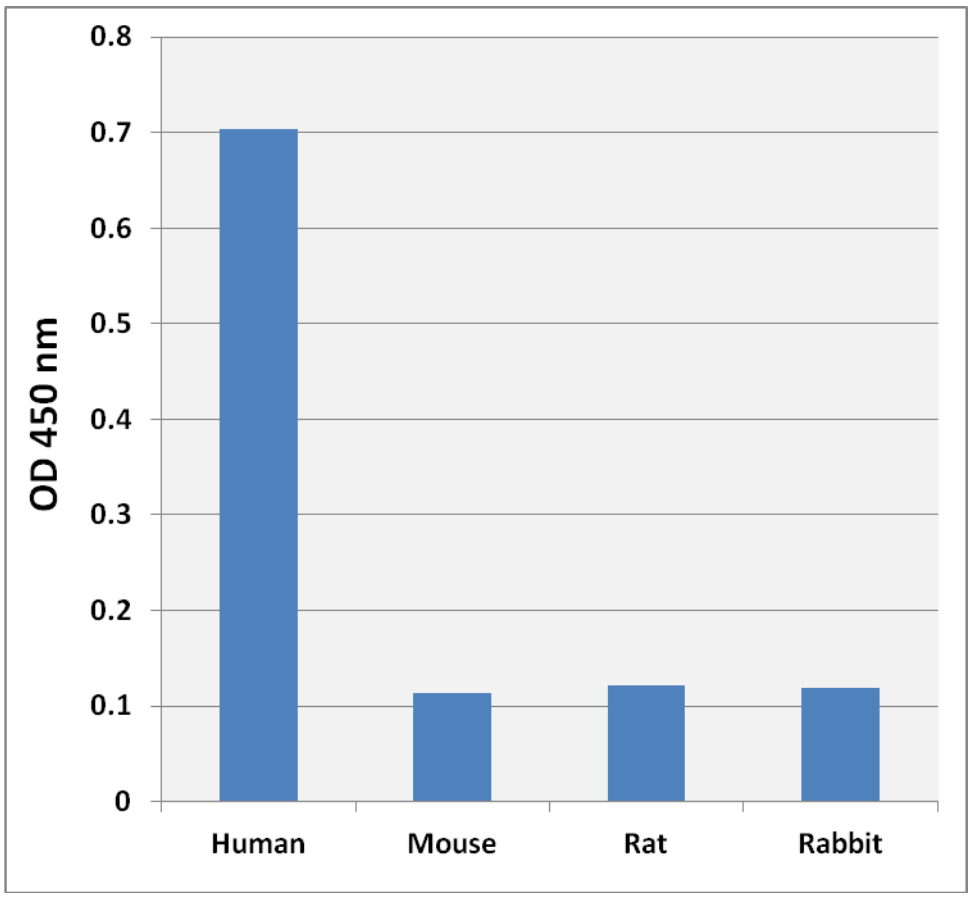

Figure 2: Detection of Haptoglobin in Serum. Each serum sample was diluted 250,000 fold according to the protocol above and then tested using the Human Haptoglobin ELISA Kit.

References

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Warranty

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