ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human Haptoglobin ELISA Kit

(Catalog Number: 31400)

For the quantitative determination of haptoglobin in serum, plasma or cell culture supernate

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

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INTRODUCTION

Human haptoglobin is an acute-phase glycoprotein produced predominantly by liver[1]. It is composed of covalently linked α subunits (M.W. 9-23 kDa) and β subunits (M.W. 35-40 kDa)[2]. Human haptoglobin gene has two alleles *Hp1* and *Hp2* that lead to the formation of HPT1-1 (covalently linked dimer by Cys15), HPT1-2 (hetero-oligomer) and HPT2-2 (oligomer)[3]. Haptoglobin can bind to free hemoglobin released from lysed erythrocytes and prevent the formation of free radical superoxide that can be formed by the reaction of oxygen and iron from hemoglobin[4]. It is also known to be involved in immune regulation and anti-inflammation[5]. Elevated amount of haptoglobin is observed during infections and inflammations, obesity, tissue damage etc[6]. Hence, haptoglobin is used as a biomarker to detect acute allograft rejection[7], proliferative diabetic retinopathy (PDR) and diabetic kidney disease (DKD)[8]. Additionally, low haptoglobin levels are mainly observed during hemolytic anemia[9].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a rabbit polyclonal antibody against human haptoglobin. Standards and samples are pipetted into the wells and any human haptoglobin present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for human haptoglobin is added to the wells. After the last wash step, an HRP substrate solution is added and color develops in proportion to the amount of human haptoglobin bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increase in absorbance is directly proportional to the amount of captured human haptoglobin, the unknown sample concentration can be extrapolated from a reference curve included in each assay.

INTENDED USE

This Human Haptoglobin ELISA kit is designed for quantification of human haptoglobin in serum, plasma and cell culture supernatant samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with polyclonal antibody against human haptoglobin
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 50 mL
4. 100×Detection antibody solution, a polyclonal antibody against human haptoglobin conjugated to horseradish peroxidase, 0.12 mL
5. Human haptoglobin standard, 100 ng of recombinant human haptoglobin, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips
2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbency at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt. Remove any unused antibody-coated strips from the microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (50 mL) with 200 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Assay buffer may be stored for up to one month at 2-8°C.

B. 1×Wash buffer

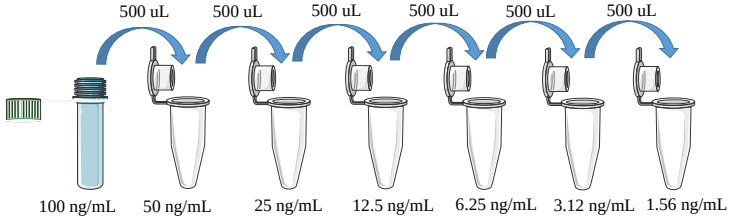
Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. 1×Wash buffer may be stored for up to one month at 2-8°C.

C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Human Haptoglobin Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 100 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 50, 25, 12.5, 6.25, 3.12, 1.56 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and stored at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **100000-fold** dilution in this assay. A three-step dilution is suggested. Step1-Add 10 µL of sample to 990 µL of 1×Assay buffer to give a 100-fold diluted sample solution. Step 2-Add 5 µL of the 100-fold diluted sample solution to 495 µL of 1×Assay buffer to give a 10000-fold diluted sample solution. Step 3-Add 50 µL of the 10000-fold diluted sample solution to 450 µL of 1×Assay buffer to give a final 100000-fold diluted sample solution. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be run in duplicate.

1. Add 100 µL of standard or sample per well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as described in step 2.
5. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 µL of Stop solution to each well, mix well by gently tapping the plate.
7. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human haptoglobin concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.

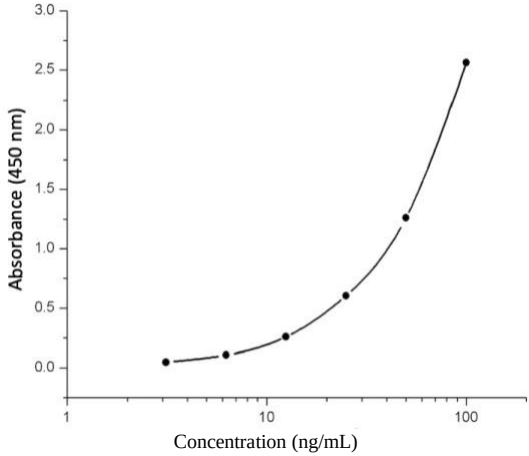
3. Determine human haptoglobin concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. A standard curve should be generated for each assay.

Human haptoglobin (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.087	0.000
3.12	0.135	0.048
6.25	0.194	0.107
12.5	0.347	0.260
25	0.692	0.605
50	1.349	1.262
100	2.650	2.563

Human haptoglobin standard curve (4-parameters)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest human haptoglobin level that can be measured by this assay is 1.56 ng/mL.

B. Precision

Intra-assay Precision (Precision within an assay) C.V. <5.63%.
Inter-assay Precision (Precision between assays) C.V. <1%.

C. Spiking

The recovery of human haptoglobin was 104.4%.

D. Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human haptoglobin were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

Sample Dilution	Absorbance (450 nm)	Concentration (ng/mL)	Recovery (%)
1:2	0.601	5.843	95.37
1:4	0.269	6.010	98.10
1:8	0.116	6.790	110.82

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SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample per well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of 1×Detection antibody solution to each well.
↓
Incubate at room temperature for 1 hour.
↓
Aspirate and wash each well three times.
↓
Add 100 µL of Substrate solution to each well.
↓
Incubate at room temperature for 15 minutes.
↓
Add 100 µL of Stop solution to each well.
↓
Measure absorbance of each well at 450 nm.
↓
Calculation