Rat Haptoglobin ELISA Kit

Enzyme Immunoassay for the quantification of Rat Haptoglobin in Plasma, Serum and Urine

Catalog number: ARG83503

Package: 96 wells

For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

As a result of hemolysis, hemoglobin is found to accumulate in the kidney and is secreted in the urine. Haptoglobin captures, and combines with free plasma hemoglobin to allow hepatic recycling of heme iron and to prevent kidney damage. Haptoglobin also acts as an Antimicrobial; Antioxidant, has antibacterial activity and plays a role in modulating many aspects of the acute phase response. Hemoglobin/haptoglobin complexes are rapidely cleared by the macrophage CD163 scavenger receptor expressed on the surface of liver Kupfer cells through an endocytic lysosomal degradation pathway. [UniProt]

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for Haptoglobin has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any Haptoglobin present is bound by the immobilized antibody. After washing away any unbound substances, a Horseradish Peroxidase (HRP)-conjugated antibody specific for Haptoglobin is added to each well and incubate. After washing away any unbound antibody-enzyme reagent, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of Haptoglobin bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of Haptoglobin in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8°C. Use the kit before expiration date.

NO	Component	Quantity	Storage information
C1	Antibody-coated microplate	8 X 12 strips	4°C
C2	Standard (Lyophilized)	1 vial	4°C
C3	5X Diluent buffer	50 ml	4°C
C4	HRP-conjugate Antibody	150 µl	4°C
C5	20X Wash buffer	50 ml	4°C
C6	TMB substrate	12 ml (ready to use)	4°C (Protect from light)
C7	STOP solution	12 ml (ready to use)	4°C
C8	Plate sealer	3 strips	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store all component in the kit at 2-8°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.

- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot & store samples at -20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Plasma- Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g. within 30 minutes of collection. Collect the supernatants and assay immediately or aliquot and store samples at-20°C up to 1 month or -80°C up to 6 months. Avoid repeated freeze-thaw cycles.

Note:

a) Do not use haemolytic, icteric or lipaemic specimens.

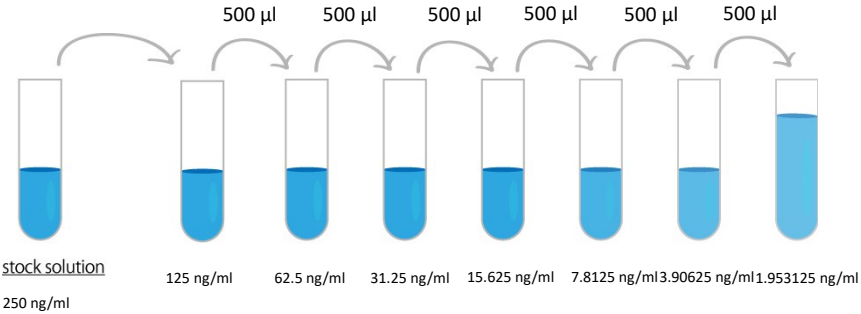
b) Samples containing sodium azide should not be used in the assay.

REAGENT PREPARATION

- 1X Wash buffer: Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer.

  *The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- 1X Diluent Buffer: Dilute 5X Diluent Buffer into distilled water to yield 1X Diluent Buffer.
- 1X HRP-conjugate Antibody: 20 minutes before use, dilute 100X HRP-conjugate Antibody into 1X Diluent Buffer to yield 1X HRP-conjugate Antibody.
- Sample: If the initial assay found samples contain Haptoglobin higher than the highest standard, the samples can be diluted with diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account.

  (It is recommended to do pre-test to determine the suitable dilution factor).
- Standards: Reconstitute the standard with 1 ml 1X diluent buffer to yield a stock concentration of 250 ng/ml. Keep the buffer in the vail for at least 15 min at RT to make sure the standard is dissolved completely before making serial dilutions. The 1X diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 125 ng/ml, 62.5 ng/ml, 31.25 ng/ml, 15.625 ng/ml, 7.8125 ng/ml, 3.90625 ng/ml, 1.953125 ng/ml. DO NOT reuse the reconstituted standard.



Dilute Haptoglobin standard as according to the table below:

Standard	Haptoglobin Conc.	µl of diluent	µl of standard
S7	125 ng/ml	500	500 (250 ng/ml Stock)
S6	62.5 ng/ml	500	500 (S7)
S5	31.25 ng/ml	500	500 (S6)
S4	15.625 ng/ml	500	500 (S5)
S3	7.8125 ng/ml	500	500 (S4)
S2	3.90625 ng/ml	500	500 (S3)
S1	1.953125 ng/ml	500	500 (S2)
S0	0	500	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT) 20 min before use.

Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100 µl of standards, samples and zero controls (1X diluent buffer) into wells, gently tap the plate to mix well. Incubate for 15 minutes at RT.
3. Aspirate each well and wash, repeating the process three times for a total 4 washes. Wash by filling each well with 1× Wash Buffer (300 µl) using a squirt bottle, manifold dispenser, or autowasher, keep the Wash Buffer in the wells for 30 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100 µl 1X HRP-conjugate Antibody into each well, gently tap the plate to mix well. Cover wells and incubate for 15 minutes at RT.
5. Aspirate each well and wash as step 3.
6. Add 100 µl of TMB Reagent to each well, gently tap the plate to mix well. Incubate for 5 minutes at RT in dark.
7. Add 100 µl of Stop Solution to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450 nm immediately. It is recommended read the absorbance within 3 min after adding STOP solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)

6. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

QUALITY ASSURANCE

Sensitivity

The minimum detectable dose (MDD) of Rat Haptoglobin ranged from 1.95-125 ng/ml. The mean MDD was 0.975 ng/ml.

Specificity

This assay recognizes natural and recombinant Rat Haptoglobin. No significant cross-reactivity or interference with the factors below was observed:

Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.