MOUSE HAPTOGLOBIN ELISA
Life Diagnostics, Inc., Catalog Number: HAPT-1

INTRODUCTION

Haptoglobin is an acute phase protein that is elevated in mouse serum due to injury, infection or disease. Studies at Life Diagnostics, Inc. and by others[1-3] indicate that haptoglobin levels may increase 10-fold or more. Haptoglobin therefore provides a useful acute phase biomarker in mice.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified mouse haptoglobin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse haptoglobin antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 30 minutes. This results in haptoglobin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If haptoglobin is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of haptoglobin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Haptoglobin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Haptoglobin stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD25-10, 25 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use estimate the final volume of diluent required for your assay and dilute one volume of the 10x stock with nine volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The standard is provided as a lyophilized stock. Reconstitute with distilled or deionized water as described on the vial label. *(the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if future use is intended)*.
2. Label 7 polypropylene microcentrifuge tubes as 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 1.95 ng/ml.
3. In the tube labeled 125 ng/ml dispense the volume of diluent detailed on the stock vial label. Then add the volume of stock, also detailed on the label, and mix. This provides the 125 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 62.5, 31.25, 15.63, 7.81, 3.91 and 1.95 ng/ml.
5. Prepare a 62.5 ng/ml standard by diluting and mixing 250 µl of the 125 ng/ml standard with 250 µl of diluent in the tube labeled 62.5 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

General Note: Haptoglobin is generally present in mouse serum at concentrations ranging from 0.1 – 2 mg/ml. To obtain values within range of the standard curve we suggest that samples be diluted 25,000-fold. The following procedure may be used for each sample to be tested:

1. Dispense 497.5 µl and 248 µl of 1x diluent into separate polypropylene tubes.
2. Pipette and mix 2.5 µl of the serum/plasma sample into the tube containing 497.5 µl of diluent. This provides a 200-fold diluted sample.
3. Mix 2.0 µl of the 200-fold diluted sample with the 248 µl of diluent in the second tube. This provides a 25,000-fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

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ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 4°C for future use.
2. Dispense 100 µl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 µl of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 30 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

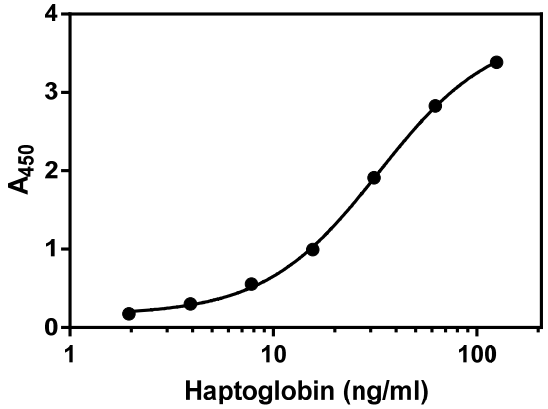
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus $\log_{10}$ of the concentration.
2. Fit the standard curve to a four-parameter logistic regression (4PL) equation (x axis = $\log_{10}$ concentration) and determine the concentration of the samples from the standard curve (remember to derive the concentration from the antilog).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum or plasma sample.
4. If the A_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with absorbance at 450nm on the Y-axis against haptoglobin concentrations on the X-axis is shown below. This curve is for illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

Haptoglobin (ng/ml)	A_{450}
125	3.384
62.5	2.828
31.25	1.911
15.63	0.995
7.81	0.554
3.91	0.303
1.95	0.176



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3. Higashisaka K. et. al. Acute phase proteins as biomarkers for predicting the exposure and toxicity of nanomaterials. Biomaterials 32:3-9 (2011).

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