RAT HAPTOGLOBIN ELISA
Life Diagnostics, Inc., Catalog Number: HAPT-2

INTRODUCTION

Haptoglobin is an acute phase protein that is elevated in rat serum due to injury, infection or disease. Measurement of haptoglobin provides a useful marker of inflammation/disease in rats[1]; levels increase up to ten-fold.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified rat haptoglobin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rat 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 rat haptoglobin stock is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 2 μg/ml rat haptoglobin stock *(the reconstituted standard remains stable for at least 10 days at 2-8°C but should be aliquoted and frozen at -20°C after reconstitution if use beyond this time is intended)*.
2. Label 7 polypropylene or glass tubes as 62.5, 31.2, 15.6, 7.8, 3.9, 1.95 and 0.98ng/ml.
3. Dispense 968.75 μl of diluent into the tube labeled 62.5 ng/ml and 300 μl of diluent into the remaining tubes.
4. Pipette 31.25 μl of the 2 μg/ml haptoglobin stock into the tube labeled 62.5 ng/ml and mix. This provides the 62.5 ng/ml haptoglobin standard.
5. Prepare a 31.25 ng/ml standard by diluting and mixing 300 μl of the 62.5 ng/ml standard with 300 μl of diluent in the tube labeled 31.25 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

Haptoglobin is present in normal rat serum at a concentration of ~ 1 mg/ml. To obtain values within range of the standard curve we suggest that samples be diluted 100,000-fold using the following procedure for each sample to be tested.

1. Dispense 998 μl and 497.5 μl of 1x diluent into separate tubes.
2. Pipette and mix 2.0 μl of the serum/plasma sample into the tube containing 998 μl of diluent. This provides a 500-fold dilution.
3. Mix 2.5 μl of the 500-fold diluted sample with 497.5 μl of diluent in the second tube. This provides a 100,000-fold dilution.

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 2-8°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.

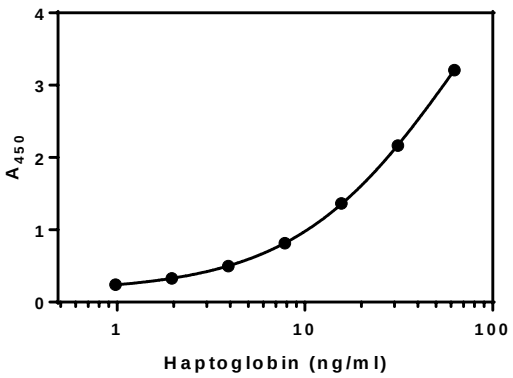
CALCULATION OF RESULTS

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}
62.5	3.210
31.25	2.165
15.63	1.365
7.81	0.814
3.91	0.497
1.95	0.329
0.98	0.243



REFERENCES

1. PS Giffen et.al., Markers of experimental acute inflammation in the Wistar Han rat with particular reference to haptoglobin and C-reactive protein. Arch Toxicol. 77:392-402 (2003)

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For technical assistance please email us at
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