

BOVINE HAPTOGLOBIN ELISA TEST KIT

Life Diagnostics, Inc., Catalog Number: 2410-7

Enzyme Immunoassay for the Quantitative Determination of Bovine Haptoglobin in Serum or Plasma

INTRODUCTION

Haptoglobin is an acute phase protein that is elevated in bovine serum as a result of injury, infection or disease. Normal serum levels of bovine haptoglobin range from ~ 25 – 50 µg/ml¹ and increase up to 50 fold during the acute phase response^{2,3}. Haptoglobin therefore serves as an excellent acute phase marker in cattle.

PRINCIPLE OF THE ASSAY

The bovine haptoglobin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-bovine haptoglobin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-bovine haptoglobin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 minutes. This results in haptoglobin molecules being sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of haptoglobin is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-bovine haptoglobin antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (lyophilized), containing 2.5 µg/ml bovine haptoglobin when reconstituted as detailed on the vial label
- Wash Buffer (10x stock, 60 ml)
- 10x Diluent (25 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional).

STORAGE OF TEST KIT AND INSTRUMENTATION

The unopened kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described above.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Serum or plasma samples should be diluted ~2,000 fold with 1x diluent in order to obtain values within the standard range.

WASH SOLUTION PREPARATION

The wash solution is provided as a 10x stock. Prior to use dilute the contents of the bottle (60 ml) with 540 ml of distilled or deionized water.

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 (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

STANDARD PREPARATION

1. The bovine haptoglobin standard is provided as a lyophilized stock. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 2.5 µg/ml bovine haptoglobin stock (*the reconstituted standard should be aliquoted and frozen at -20°C within 2 hours of reconstitution if additional use is intended*).
2. Label 7 polypropylene or glass tubes as 250, 125, 62.5, 31.25, 15.6, 7.8, and 0 ng/ml.
3. Dispense 450 µl of 1x diluent into the tube labeled 250 ng/ml and 300 µl of 1x diluent into the remaining tubes.
4. Pipette 50 µl of the 2.5 µg/ml haptoglobin standard into the tube labeled 250 ng/ml and mix. This provides the working 250 ng/ml haptoglobin standard.
5. Prepare a 125 ng/ml standard by diluting and mixing 300 µl of the 250 ng/ml standard with 300 µl of 1x diluent in the tube labeled 125 ng/ml. Similarly prepare the 62.5, 31.25, 15.6, and 7.8 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: Haptoglobin is present in normal bovine serum at a concentration of 25 - 50 µg/ml and can increase to over 1 mg/ml. In order to obtain values within the range of the standard curve, we suggest that samples initially be diluted 2,000 fold using the following procedure for each sample to be tested:

1. Dispense 195 μl and 392 μl of 1x diluent into separate tubes.
2. Pipette and mix 5 μl of the serum/plasma sample into the tube containing 195 μl of diluent. This provides a 40 fold diluted sample.
3. Mix 8 μl of the 40 fold diluted sample with the 392 μl of diluent in the second tube. This provides a 2,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 μl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This should preferably be performed using a plate washer (400 μl /well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
7. Add 100 μl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 30 minutes.
9. Wash as detailed in 4 to 6 above.
10. Dispense 100 μl of TMB Reagent into each well.
11. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
12. 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 the optical density at 450 nm with a microtiter plate reader within 15 minutes. ***In the event that the standards or samples exceed the absorbance range of your plate reader, optical density may be determined at 405 nm.***

CALCULATION OF RESULTS

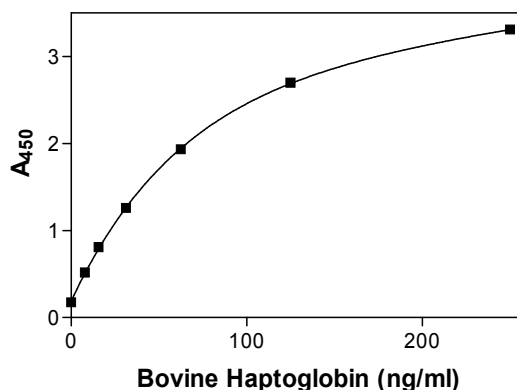
1. Calculate the average absorbance values (A_{450}) for each set of reference standards, and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of haptoglobin in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of haptoglobin in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve when tested at a dilution of 2,000, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y axis against haptoglobin concentrations on the X axis is shown below. This curve is for the purpose of 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)	Absorbance (450 nm)
250	3.308
125	2.697
62.5	1.931
31.25	1.258
15.6	0.805
7.8	0.515
0	0.173

Representative Bovine Haptoglobin Standard Curve



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

REFERENCES

1. SM Salonen et.al., Quantitative determination of bovine serum haptoglobin in experimentally induced Escherichia coli mastitis. Res Vet Sci. 60:88-91 (1996)
2. M Morimatsu et.al., Bovine Haptoglobin: single radial immunodiffusion assay of its polymeric forms and dramatic rise in acute-phase sera. Vet Immunol Immunopathol. 33:365-372 (1992)
3. DL Godson et.al., Serum haptoglobin as an indicator of the acute phase response in bovine respiratory disease. Vet Immunol Immunopathol. 51:277-92 (1996).