

# PIG HAPTOGLOBIN ELISA

## Life Diagnostics, Inc., Catalog Number: HAPT-9

### INTRODUCTION

Haptoglobin is an acute phase protein that is elevated in pig serum because of injury, infection or disease. Measurement of haptoglobin therefore provides a convenient biomarker of inflammation and disease in pigs.<sup>1-5</sup>

### PRINCIPLE OF THE ASSAY

The assay uses affinity purified pig haptoglobin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated pig haptoglobin antibodies for detection. Serum or plasma samples are first denatured and subsequently diluted. Standards and diluted samples are then incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. 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-conjugate. 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. 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
- Denaturing buffer: HD25-1, 25 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°C to 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 pig haptoglobin stock is provided lyophilized. Add the volume of distilled or deionized water indicated on the vial label and mix gently until dissolved to obtain a 300 µg/ml stock (***the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if future use is intended***).
2. Dilute 10 µl of the reconstituted 300 µg/ml pig haptoglobin with 190 µl of denaturing buffer. Incubate at room temperature for at least 10 minutes.
3. Dilute 20 µl of the denatured haptoglobin with 0.98 ml of 1x diluent. This provides the 300 ng/ml standard.
4. Label 6 polypropylene or glass tubes as 150, 75, 37.5, 18.75, 9.38 and 4.69 ng/ml.
5. Dispense 300 µl of diluent into the labelled tubes.
6. Prepare a 150 ng/ml standard by diluting and mixing 300 µl of the 300 ng/ml standard with 300 µl of diluent in the tube labeled 150 ng/ml. Similarly prepare the remaining standards by serial dilution.

### SAMPLE PREPARATION

Haptoglobin is present in normal pig serum at a concentration of ~ 1 mg/ml. To obtain values within the range of the standard curve we suggest that samples be diluted 8,000-fold using the following procedure.

1. Dispense 190 µl of denaturing buffer and 997.5 µl of 1x diluent into separate tubes.
2. Pipette and mix 10 µl of the serum/plasma sample into the tube containing 190 µl of denaturing buffer. This provides a 20-fold diluted/denatured sample.
3. Mix 2.5 µl of the 20-fold diluted/denatured sample with the 997.5 µl of 1 x diluent in the second tube. This provides an 8,000-fold dilution of the sample.

### 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°C to 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  $\mu$ l/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100  $\mu$ l of HRP-conjugate into each well.
7. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100  $\mu$ l 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  $\mu$ l 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.

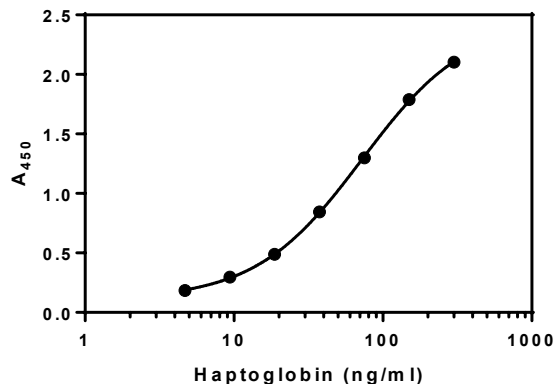
### CALCULATION OF RESULTS

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.

Haptoglobin (ng/ml)	$A_{450}$
300	2.103
150	1.789
75	1.298
37.5	0.844
18.75	0.488
9.38	0.296
4.69	0.184



### REFERENCES

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2. Enemark HL, et al. Pathogenicity of *Cryptosporidium parvum* – evaluation of an animal infection model. *Vet Parasitol.* 113:35-57 (2003)
3. Kreuzer S et. al. No beneficial effects evident for *Enterococcus faecium* NCIMB 10415 in weaned pigs infected with *Salmonella enterica* Typhimurium DT104. *Appl Environ Microbiol.* 78(14):4816-4825
4. Pomorska-Mol M, Markowska-Daniel I and Kwit K. Acute phase protein response in pigs experimentally co-infected with swine influenza virus and *Bordetella bronchiseptica*. *Centr Eur J Immunol.* 37(3):221-226 (2012)
5. Pomorska-Mol M, Kwit K and Markowska-Daniel I. Major acute phase proteins in pig serum from birth to slaughter. *Bull Vet Inst Pulawy.* 56:553-557 (2012)

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