

CHICKEN HEMOPEXIN ELISA

Life Diagnostics, Inc., Catalog Number: HPX-5

INTRODUCTION

Hemopexin (Hpx) is an acute phase protein of approximately 60 kDa. It is synthesized in the liver and circulates in blood. Grieninger et.al., reported that plasma levels increase approximately five-fold during the acute phase response in chickens (ref 1). In studies at Life Diagnostics, we found that levels increase up to 25-fold. We also found that samples with elevated Hpx also had increased levels of the acute phase proteins alpha-1-acid glycoprotein and serum amyloid A.

PRINCIPLE OF THE ASSAY

The assay uses two chicken Hpx antibodies; one for solid-phase immobilization (microtiter wells) and one, conjugated to horseradish peroxidase (HRP), 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 45 minutes. This results in Hpx molecules being sandwiched between the immobilization and detection antibodies. The wells are washed to remove unbound HRP-conjugate and TMB is added and incubated for 20 minutes. If Hpx 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 Hpx is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Hpx antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Hpx 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
- Microcentrifuge 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 4°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 Hpx stock is provided lyophilized. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved (***the reconstituted standard remains stable for at least 1 day at room temperature but should be frozen at -20°C after reconstitution if future use is intended***).
2. Label seven microcentrifuge tubes as 100, 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
3. In the tube labeled 100 ng/ml prepare the 100 ng/ml standard as detailed on the stock vial label.
4. Dispense 250 µl of diluent into the tubes labeled 50, 25, 12.5, 6.25, 3.13 and 1.56 ng/ml.
5. Prepare the 50 ng/ml standard by mixing 250 µl of the 100 ng/ml standard with 250 µl of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We found that Hpx is present in chicken plasma at concentrations of 0.18 to 4.4 mg/ml. To obtain values within the range of the standard curve, and to eliminate matrix effects, plasma samples should be diluted at least 20,000-fold (optimal dilution factors should be determined by the end user). A 20,000-fold dilution can be obtained as follows:

1. Dispense 495 µl and 497.5 µl of 1x diluent into separate tubes.
2. Pipette and mix 5 µl of the plasma sample into the first tube containing 495 µl of diluent. This provides a 100-fold diluted sample.
3. Mix 2.5 µl of the 100-fold diluted sample with the 497.5 µl of diluent in the second tube. This provides a 20,000-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 4°C for future use.

2. Dispense 100 μ l 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 μ l/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μ 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 μ 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 μ 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.

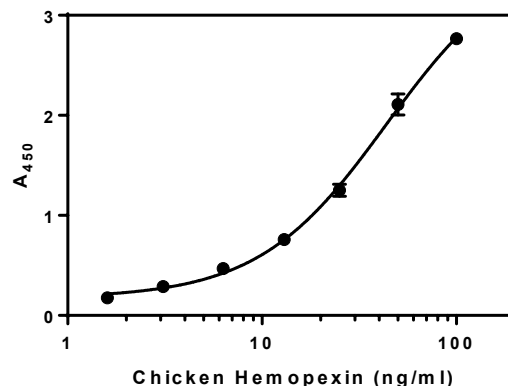
CALCULATION OF RESULTS

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 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 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.

Hpx (ng/ml)	Absorbance (450 nm)
100	2.766
50	2.109
25	1.250
12.5	0.758
6.25	0.468
3.13	0.288
1.56	0.177



REFERENCES

1. Grieninger G. et.al. Hemopexin is a developmentally regulated, acute phase plasma protein in the chicken. J. Biol. Chem. 33:15719-15724 (1986)

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For technical assistance please email us at techsupport@lifediagnosics.com