

MOUSE HEMOPEXIN ELISA

Life Diagnostics, Inc., Catalog Number: HPX-1

INTRODUCTION

Hemopexin is an acute phase protein that is elevated up to 4-fold in mouse serum and plasma because of inflammation and infection.^{1,2} Hemopexin provides a convenient marker of inflammation and tissue injury in mice.

PRINCIPLE OF THE ASSAY

The assay uses affinity purified mouse hemopexin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated mouse hemopexin 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 45 minutes. This results in hemopexin 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 hemopexin 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 hemopexin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Hemopexin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Hemopexin 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 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 stock is provided lyophilized. Reconstitute with distilled or deionized water as described on the vial label (*the reconstituted standard should be aliquoted and frozen at -20°C if future use is intended*).
2. Label 6 polypropylene microcentrifuge tubes as 100, 50, 25, 12.5, 6.25, and 0 ng/ml
3. Dispense 950 μ l of 1x diluent into the tube labeled 100 ng/ml and 300 μ l of 1x diluent into the remaining tubes.
4. Pipette 50 μ l of the 2 μ g/ml hemopexin standard into the tube labeled 100 ng/ml and mix. This provides the 100 ng/ml standard.
5. Prepare the 50 ng/ml standard by diluting and mixing 300 μ l of the 100 ng/ml standard with 300 μ l of diluent in the tube labeled 50 ng/ml.
6. Similarly prepare the 25, 12.5, and 6.25 ng/ml standards by serial dilution.

SAMPLE PREPARATION

We found that hemopexin was present in mouse serum at concentrations ranging from 0.2 – 1 mg/ml. To obtain values within range of the standard curve, samples should be diluted 25,000-fold. We suggest the following procedure for each sample to be tested.

1. Dispense 497.5 μ l and 248 μ l of 1x diluent into separate polypropylene tubes.
2. Pipette and mix 2.5 μ l of the serum/plasma sample into the tube containing 497.5 μ l of diluent. This provides a 200-fold dilution.
3. Mix 2.0 μ l of the 200-fold diluted sample with the 248 μ l of diluent in the second tube. This provides a 25,000-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 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.

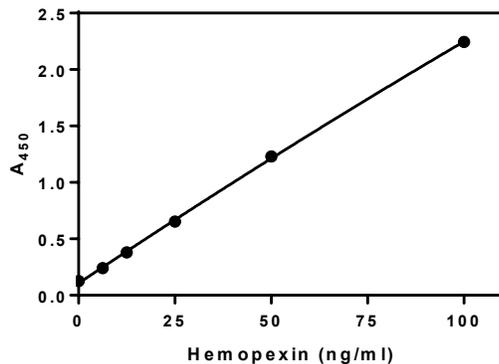
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum 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 450 nm on the Y-axis against hemopexin concentrations on the X-axis is shown below. This curve is for illustration only.

Hemopexin (ng/ml)	A_{450}
100	2.245
50	1.230
25	0.653
12.5	0.380
6.25	0.241
0	0.125



REFERENCES

1. X Duan, et al. Immunodepletion of albumin for two-dimensional gel detection of new mouse acute-phase protein and other plasma proteins. *Proteomics*. 5:3991-4000 (2005)
2. R Wait, et al. Reference maps of mouse serum acute-phase proteins: Changes with LPS-induced inflammation and apolipoprotein A-I and A-II transgenes. *Proteomics*. 5:4245-4253 (2005)

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