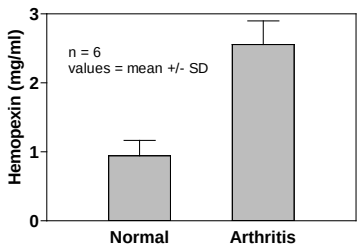
RAT HEMOPEXIN ELISA
Life Diagnostics, Inc., Catalog Number: HPX-2

INTRODUCTION

Hemopexin is an acute phase protein that is elevated up to 3-fold in rat serum and plasma because of inflammation.[1,2] As illustrated in the figure below, studies at Life Diagnostics, Inc. indicated that hemopexin was elevated approximately 2.5-fold in serum of arthritic rats.

Serum Hemopexin in a Rat Arthritis Model



PRINCIPLE OF THE ASSAY

The assay uses affinity purified rat hemopexin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rat 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, and 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 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 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 7 polypropylene microcentrifuge tubes as 250, 125, 62.5, 31.25, 15.6, 7.8 and 3.9 ng/ml
3. Dispense 875 µl of 1x diluent into the tube labeled 250 ng/ml and 300 µl of diluent into the remaining tubes.
4. Pipette 125 µl of the 2 µg/ml hemopexin standard into the tube labeled 250 ng/ml and mix. This provides the 250 ng/ml hemopexin standard.
5. Prepare a 125 ng/ml standard by diluting and mixing 300 µl of the 250 ng/ml standard with 300 µl of diluent in the tube labeled 125 ng/ml.
6. Similarly prepare the remaining standards by two-fold serial dilution.

SAMPLE PREPARATION

We found that hemopexin was present in rat serum at concentrations ranging from 1-3 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 µl and 248 µl of 1x diluent into separate polypropylene tubes.
2. Pipette and mix 2.5 µl of the serum/plasma sample into the tube containing 497.5 µl of diluent. This provides a 200-fold dilution.
3. Mix 2.0 µl of the 200-fold diluted sample with the 248 µl of diluent in the second tube. This provides a 25,000-fold dilution.

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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 4ºC 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 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 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.

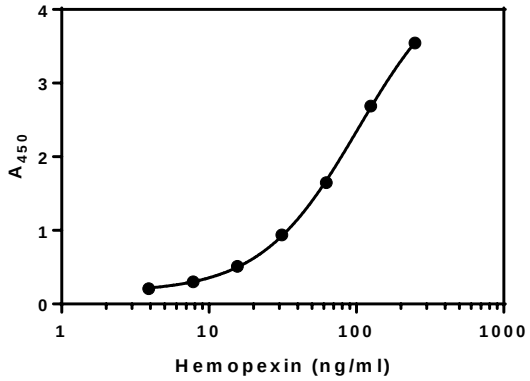
CALCULATION OF RESULTS

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 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}
250	3.546
125	2.687
62.5	1.649
31.25	0.938
15.6	0.512
7.8	0.301
3.9	0.210



REFERENCES

1. I Miller, et al. Proteins of rat serum: III. Gender-related differences in protein concentration under baseline conditions and upon experimental inflammation as evaluated by two-dimensional electrophoresis. Electrophoresis. 20:836-845 (1999)
2. S Marainkovic, et al. IL-6 modulates the synthesis of a specific set of acute phase plasma proteins in vivo. J Immunol. 142:808-812 (1989)

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