ELISA kits available from ADI:

Human: Adiponectin (Acrp30 and gAcrp30), Albumin, Aldosterone, AFP, beta-amyloid 1-40/42, Angiogenin, Angiopoietin-2, beta-2M, BMP-7, C-peptide, CRP, Cox-2, Ferritin, PSA, fPSA, GH, IgG, IgM, IgE, IgG1, IgG4, Insulin, NSE, CA125, CA199, CA242, PAP, Resistin, SHBG, LH, FSH, TSH, T3, T4, and Steroid ELISA kits (cortisol, estradiol, testosterone, progesterone).

Monkey: IgM, IgG, IgA, IgE

Rat: Albumin, CRP, IgG, IgM, Alpha-1- Acid glycoprotein

Mouse: Albumin, IgA, IgG, IgG1, IgG2a, IgG2b, IgG3, IgE, IgM, Leptin, Resistin, Acrp30, CRP, Haptoglobin, TNF-alpha
Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin

T**urkey**: IgG

Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin

Pig: Albumin, IgG, IgM

Dog: CRP, IgG, IgM

Cat: IgG, IgM

Goat: IgG

Rabbit: CRP, IgG

Sheep: IgG

Instruction Manual No. M-610-710-HPX

Rat Hemopexin (HPX)

ELISA KIT Cat. #. 610-710-HPX

For Quantitative Determination of Hemopexin in Rat Serum

ALPHA DIAGNOSTIC INTERNATIONAL

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DRAFT MANUAL: PLEASE CONSULT THE MANUAL SUPPLIED WITH THE KIT FOR ANY LOT SPECIFIC CHNAGES.

Rat HemopexinELISA KIT Cat. # 610-710-HPX

Kit Components, 96 tests	Cat #
Anti-Rat Hemopexin coated strip plate (8 wells x 12 strips)	610-711
Rat Hemopexin Reference Standard (2 ug/ml), lyophilized, *Reconstitute with dH_20 according to vial label*	610-712
Anti- Hemopexin-HRP Conjugate, 11 ml	610-713
10x Sample Diluent, 25 ml	SD-10L
Wash Buffer (20x), 50 ml	WB-20
TMB Substrate, 11 ml	81091
Stop solution, 11 ml	81101
Instruction Manual	M-6490

NTRODUCTION

Hemopexin, a globulin (beta-glycoprotein) synthesized by liver, accounts for about 1.4% of total serum protein. Like albumin, it binds heme with high affinity and transports it to liver for salvage of the iron. Human Hemopexin consists of a single polypeptide chain of 439 amino acids (~50-57 kda, carbohydrate ~23%; plasma concn in human 0.8-1 mg/ml). Structurally Hemopexin consists of two similar halves of approximately two hundred amino acid residues connected by a histidine rich hinge region. Each half is itself formed by the repetition of a basic unit of some 35 to 45 residues. Hemopexin has been found in the serum of all mammals studied and it is polymorphic in rabbits and swine. Hemopexin is an acute phase protein that is elevated 2-3 fold in rat serum and plasma as a result of inflammation and arthritis. Hemopexin provides a convenient marker of inflammation and tissue injury in the rat.

Recent studies have demonstrated that Hemopexin acts as an extracellular antioxidant against hemoglobin-mediated damage in inflammation. Hemopexin protects against heme toxicity and conserves and recycles iron. Abnormal levels of Hemopexin are associated with hemolytic anemia, chronic neuromuscular disease, and acute intermittent porphyria.

Alpha Diagnostic Intl's rat Hemopexin ELISA kit is a highly sensitive sandwich type assay for the measurement of Hemopexin in serum. The assay can be adapted to measure rat Hemopexin in other biological fluids such as plasma and urine, and in culture medium.

DILUTION OF SAMPLES

Samples containing more than 250 ng/ml rat HEMOPEXIN should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor. It is possible to use normal saline or PBS for sample dilution if larger volumes of samples are taken for dilution or if more sample diluent is required.

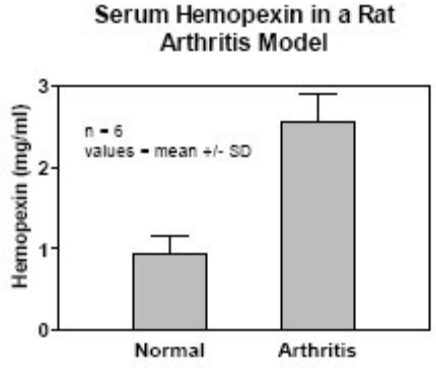
CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on a graph paper by plotting net absorbance values of standards against appropriate HEMOPEXIN concentrations. Read off the HEMOPEXIN concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:25,000 then the values must be multiplied by 25,000 and results are expressed as mg/ml.

PERFORMANCE CHARACTERISTICS

Detection Limit: The minimum rat HEMOPEXIN concentration detectable using this assay is below 1.5 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Rat HEMOPEXIN levels in serum may vary from 1-3 ug/ml to above in normal animals and increase in inflammation, acute phase response and arthritis. Each laboratory should establish testing ranges for the animal population being investigated.



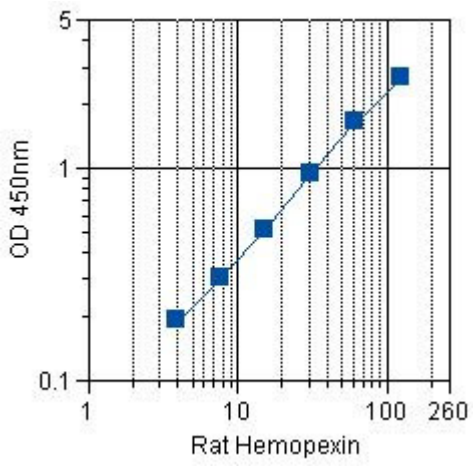
Specificity: The antibodies used in this kit are specific for rat hemopexin with no cross-reactivity with other rat serum proteins.

Species Crossreactivity: Utility of the kit to detect hemopexin form other species (crossreactivity) has not studied.

Work Sheet of Typical Assay-Rat Hemopexin

Wells	Stds/samples		Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Standard A**	0.0 ng/ml	0.106	
B1, B2	**Standard B**	3.9 ng/ml	0.210	
C1, C2	**Standard C**	7.8 ng/ml	0.301	
D1, D2	**Standard D**	15.6 ng/ml	0.510	
E1, E2	**Standard E**	31.25 ng/ml	0.940	
F1, F2	**Standard F**	62.5 ng/ml	1.65	
F1, F2	**Standard G**	125 ng/ml	2.70	
F1, F2	**Standard F**	250 ng/ml	3.54	
G1, G2	**Sample 1** 1:2500 dilution		0.98	32.1 ng/ml

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



A typical assay Standard Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Rat Hemopexin ELISA kit is based on binding of Rat HEMOPEXIN from samples to two antibodies, one immobilized on the microtiter well plates, and other conjugated to the enzyme horseradish peroxidase. After a washing step, chromogenic substrate is added and colors developed. The enzymatic reaction (color) is directly proportional to the amount of HEMOPEXIN present in the sample. Adding stopping solution terminates the reaction. Absorbance is then measured on a microtiter well ELISA reader at 450 nm. and the concentration of HEMOPEXIN in samples and control is read off the standard curve.

MATERIALS AND EQUIPMENT REQUIRED

Adjustable micropipet (5-1000 ul) and multichannel pipet with disposable plastic tips. Reagent troughs, plate washer (recommended) and ELISA plates Reader.

PRECAUTIONS AND SAFETY INSTRUCTIONS

The Rat HEMOPEXIN ELISA Kit is for research use only.

Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SPECIMEN COLLECTION AND HANDLING

Collect blood by venipuncture, allow clotting, and separating the serum by centrifugation at room temperature. Do not heat inactivate the serum. If sera can not be immediately assayed , store frozen for up to six months. Avoid repeated freezing and thawing of samples. It is also possible to use plasma for testing.

REAGENT PREPARATION

1. Dilute the Sample Diluent 1:10 with water (10 ml diluent in 90ml water). Dilute only the required reagent. Store diluted solution at 2-8^{o} C for 3-4 days.

2. The Wash Buffer is a 20x stock. Dilute the entire 50 ml with distilled or deionized water to 1 L total volume. Store at room temperature for the entire use of the kit.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the label. After opening the kit components, the shelf life is approximately 2 months.

TEST PROCEDURE *(ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).*

1. Reconstitute the lyophilized Reference Standard with the amount of distilled water indicated on the vial label. The stock concentration will be 2 ug/ml. Store unused Reference Standard at -20°C.

2. Prepare liquid standards series using the following dilution scheme:

		Diluent	Stds Final Concn	Final Volume
Concn	Volume			
Stock 2000 ng/ml	125 uL	+ 875 uL	**H** 250 ng/ml	1000 uL
Std. H	500 uL	+ 500 uL	**G** 125 ng/ml	1000 uL
Std. G	500 uL	+ 500 uL	**F** 62.5 ng/ml	1000 uL
Std. F	500 uL	+ 500 uL	**E** 31.25 ng/ml	1000 uL
Std. E	500 uL	+ 500 uL	**D** 15.6 ng/ml	1000 uL
Std. D	500 uL	+ 500 uL	**C** 7.8 ng/ml	1000 uL
Std. C	500 uL	+ 500 uL	**B** 3.9 ng/ml	1000 uL
		+ 500 uL	**A** 0 ng/ml	500 uL

Diluted standards should be prepared before use in required volumes and the unused diluted standards used within 1-2 days.

Sample Dilutions

Hemopexin is generally present in rat serum at concentrations ranging from 1-3 mg/ml. In order to obtain values within the range of the standard curve samples should be diluted 25,000 fold. We suggest the following procedure for each sample to be tested:

i) Prepare 1:100 sample dilution (5 ul sample +495 diluent)

ii) prepare 1:1000 sample dilution (take 5 ul of 1:100 and 495 ul diluent); final 1:1000

iii) prepare 1:25,000 sample dilution (20 ul of 1:1000 and 480 ul diluent); final dilution 1:25,000 (volume 125 ul). you will need 200 ul of each sample for a single test (100 ul/sample in duplicate).

Label or mark the microtiter well strips to be used on the plate.

3. Pipet **100 ul standards and diluted samples** (duplicate) into appropriate wells.

4. Mix gently, and incubate at room temperature (20-25oC) **for 60 minutes on a plate shaker (100-150 rpm).** If a shaker is not available then the plates can be gently mixed for 5-10 secs every 15-20 min. This may decrease the overall reaction but the incubation time may be increased by 15-30 min to achieve highest stds reading of >2.00.

5. **Wash the wells 5 times** with 300 ul of 1x wash buffer using an automated ELISA washer. IF washing manually (using a multi-channel pipette or squirt bottle) then strike the wells on a paper towel or adsorbent paper between each washing cycle.

6. Pipette **100 ul of Ab-enzyme conjugate** into each well. Mix gently, and incubate for **45 minutes at room temperature on a plate shaker (100-150 rpm).** If a shaker is not available then the plates can be gently mixed for 5-10 secs every 5 min. This may decrease the overall reaction but the incubation time may be increased by 15-30 min to achieve highest stds reading of >2.00.

7. Add **100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes at room temperature**. Blue color develops.

8. Stop the reaction by adding 100 ul of stop solution to all wells. Mix gently. Blue color turns yellow.

9. Measure the absorbance at 450 nm using an ELISA reader. Color is stable for at least 30 minutes after stopping.

NOTES: Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 2-8^{o}C. Addition of the HRP substrate solution starts a kinetic reaction, which is terminated by dispensing the stopping solution. Therefore, keep the incubation time for each wells the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.