ELISA kits available from ADI:

Catalog#	ProdDescription
600-400-CTN	Dog Cardiac Troponin 1 (Tn-I) ELISA Kit
600-420-CTN	Monkey Cardiac Troponin 1 (Tn-I) ELISA Kit
600-430-MTN	Monkey Skeletal Muscle Troponin 1 (Tn-I) ELISA
600-440-CTN	Mouse Cardiac Tn-I ELISA kit for plasma samples
600-450-CTN	Mouse Cardiac Tn-I ELISA kit for serum samples
600-460-MTN	Mouse Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-470-CTN	Pig Cardiac Troponin 1 (Tn-I) ELISA Kit
600-480-CTN	Rabbit Cardiac Troponin 1 (Tn-I) ELISA Kit
600-510-MTN	Rat Skeletal Muscle Troponin 1 (Tn-I) ELISA Kit
600-600-DMY	Dog Myoglobin ELISA Kit
600-610-HMY	Human Myoglobin ELISA Kit
600-620-MMY	Monkey Myoglobin ELISA Kit
600-630-MMY	Mouse Myoglobin ELISA Kit
600-640-PMY	Pig Myoglobin ELISA Kit
600-650-RMY	Rabbit Myoglobin ELISA Kit
600-660-RMY	Rat Myoglobin ELISA Kit

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, Troponin-I, TNF-alpha
Autoimmune Antibody detection kits for ANA, ssDNA, dsDNA, Histone, Sm, RNP, SSA, SSB, Scl70, Ovalbumin, Cardiolipin, CIC

Chicken: IgG, IgM, IgY, Ovalbumin **Turkey**: IgG
Bovine: Albumin, IgG, IgM, Lactoferrin, Transferrin
Pig: Albumin, IgG, IgM **Dog:** CRP, IgG, IgM
Cat: IgG, IgM **Sheep:** IgG **Goat:** IgG **Rabbit**: CRP, IgG
See Details at the web site or Contact ADI

Instruction Manual No. M-600-700-HPX

Mouse Hemopexin

ELISA KIT Cat. # 600-700-HPX

For Quantitative Determination of Mouse Hemopexin in Serum or Plasma

For In Vitro Research Use Only

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Mouse Hemopexin ELISA KIT
Cat. No. 600-700-HPX

Kit Components, 96 tests	Cat #
Anti-Mouse hemopexin antibody coated strip plate (8 wells x 12 strips), #600-700-1	1 plate
Mouse hemopexin **Reference Standard** 2 ug/ml, **lyophilized**. *Reconstitute with dH_20 volume specified on the vial,* #600-700-2	1 vial
Sample Diluent (10X), 25 ml #600-700-SD	1 bottle
Anti-Mouse hemopexin HRP Conjugate 11 ml, #600-700-3	1 bottle
Wash Buffer (100X) 10 ml, #600-700-WB (dilute 1:100 with distilled water)	1 bottle
TMB Substrate Solution, 11 ml, #600-700-TMB	1 bottle
Stop solution, 11 ml, #600-700-SS	1 bottle
Instruction Manual, # M-600410	1 manual

INTRODUCTION

Hemopexin (or haemopexin; HPX), also known as beta-1B-glycoprotein is a protein that in humans is encoded by the HPX gene[1][2][3] and belongs to hemopexin family of proteins. Hemopexin binds heme with the highest affinity of any known protein. Its function of scavenging the heme released or lost by the turnover of heme proteins such as hemoglobin and thus protects the body from the oxidative damage that free heme can cause. In addition, hemopexin releases its bound ligand for internalization upon interacting with a specific receptor situated on the surface of liver cells. This function of hemopexin is to preserve the body's iron. Its levels in serum reflect how much heme is present in the blood. Therefore, low hemopexin levels indicates that there has been significant degradation of heme containing compounds and hemopexin is made to scavenge any heme it can. Low hemopexin levels are one of the diagnostic features of a hemolytic anemia. Hemopexin is an acute phase protein that is elevated in mouse serum and plasma as a result of inflammation and infection. The level of induction may be as much as 3-4 fold. Hemopexin therefore provides a convenient marker of inflammation and tissue injury in the mouse.

ADI's Mouse hemopexin ELISA provides is a rapid, specific and sensitive assay for Quantitative Determination of Mouse Hemopexin in Serum or Plasma.

STANDARD PREPARATION

1. Add the volume of distilled or de-ionized water indicated on the lyophilized mouse hemopexin standard vial label to the standard vial and mix gently until dissolved. This provides a 2 ug/ml stock (the reconstituted standard should be aliquoted and frozen at -20oC after reconstitution 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 ul of 1x diluent into the tube labeled 100 ng/ml and 300 ul of diluent into the remaining tubes.

4. Pipette 50 ul of the 2 ug/ml hemopexin standard into the tube labeled 100 ng/ml and mix. This provides the working 100 ng/ml hemopexin standard.

5. Prepare a 50 ng/ml standard by serial diluting and mixing 300 ul of the 100 ng/ml standard with 300 ul of diluent in the tube labeled 50 ng/ml. Similarly prepare the 25, 12.5, and 6.25 ng/ml standards by serial dilution.

Note: Do not store diluted standards and prepare fresh standards from the stock.

SAMPLE PREPARATION

General Note: Hemopexin is generally present in mouse serum at concentrations ranging from 0.2 – 1 mg/ml. In order to obtain values within the range of the standard curve samples should be diluted 10,000-30,000 fold. We suggest the following procedure for each sample to be tested:

1. Dilute samples 1:200 (2.5 ul sample into 497.5 ul of 1X diluent). Mix it thoroughly for 5-10 seconds or vortex.

2. Dilute the above 1:200 by taking 2.0 ul and 248 ul of the 1X diluent. This provides a 200 fold diluted sample or a total dilution of 1:25,000.

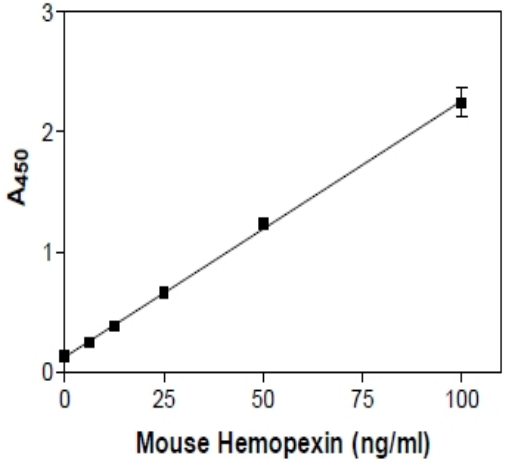
Note: We recommend using at least 2.5 ul sample for dilution but if more sample is available then it is better to dilute 5 or 10 ul sample in the first step or do the dilutions in 3-steps. This will improve accuracy of the sample. If the above scheme is followed then you will need about 750-ul 1X diluent per sample. If necessary all samples can be diluted in PBS, pH 7.4.

References: Takahashi M (1985) PNAS 82, 73-77; Smith A (1993) JBC 268, 7365-7771; Miller YI (1996) Biochem. 35, 13112-13117; Shipulina N (2001) J. Protien Chem. 19, 239-248.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn
A1, A2	100 ng/ml	2.245	
B1, B2	50 ng/ml	1.230	
C1, C2	25 ng/ml	0.653	
D1, D2	12.5 ng/ml	0.380	
E1, E2	6.25 ng/ml	0.241	
F1, F2	0 ng/ml	0.125	

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 Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on linear graph paper, with absorbance values on vertical or Y-axis & concentrations on the horizontal or X-Axis. Multiply the derived concentrations by the dilution factor to determine the actual concentration of hemopexin in the serum/plasma sample.

If available, graphing software may be used to analyze the data. Depending on the range of the standard curve used, we find that good fits of the data may be obtained with linear regression analysis or using a two-site binding model. Alternatively, standard curves may be generated using a point-to- point fit.

PRINCIPLE OF THE TEST

The mouse hemopexin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-mouse hemopexin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-mouse hemopexin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and 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-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of hemopexin is proportional to the optical density of the test sample.

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 mouse hemopexin ELISA Kit is for research use only.
Caution: This kit contains human material. The source material used for manufacture of this component tested negative for HbsAg, HIV1/2 and HCV by FDA-approved methods. However, all human blood samples should be considered potentially infectious and appropriate precautions should be taken in handling these specimens.

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 cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. Avoid grossly hemolytic, lipemic or turbid samples.

Reagent Preparation:

Dilute wash buffer (1:100) with distilled water (10 ml stock in total of 990 mL). Store at 4oC.

Sample Diluent: Dilute 10X stock with distilled water eg: **10 ml** stock in 90 ml of dH2O or 25 ml stock in 225 ml of distilled water.

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.

DILUTION OF SAMPLES

Samples containing more than 100 ng/ml or highest standard should be further diluted and re-tested. The results obtained should be multiplied by the appropriate dilution factor.

REAGENT PREPARATION & TEST PROCEDURE

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

Label or mark the microtiter well strips to be used on the plate. Prepare the standards from the stock according to instructions on page 6.

1. Reconstitute the lyophilized Reference Standards with 0.963 ml of distilled water or volume specified on the vial label and other dilution instructions provided on the vial. Mix gently for 5-10 min at room temp. Store unused Reference Standards in aliquots at -20°C. They are stable for at least 1 month at -20oc and 6 months at -70oC. Do not freeze and thaw the standards.

2. Pipet **100 ul diluted standards and diluted samples** into appropriate wells. Mix gently for 5-10 seconds, and **incubate on an orbital plate (100-150 rpm) shaker at room temperature (18-25oC) for 45 minutes**.

3. Remove or aspirate the plate contents and **wash the wells 3 times** with 400 ul of wash buffer using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add wash buffer, shake the contents of 5-10 seconds and repeat the steps. Tap the plate over fresh paper towels between each washing.

4. Pipette **100 ul of mouse hemopexin-HRP conjugate** into each well, Mix gently for 5-10 seconds, and **incubate on an orbital plate (100-150 rpm) shaker at room temperature (18-25oC) for 45 minutes**.

5. Remove or aspirate the plate contents and **wash the wells 3 times** with 400 ul of wash buffer same as in step 3.

6. **Add 100 ul of TMB Substrate** into each well. Mix gently for 5-10 seconds. Cover the plate and **incubate on an orbital plate shaker for 20 minutes at room temperature (18-25oC). Blue color** develops. This step can be reduced or increased by ± 5 minutes to keep the color within reading range. If your ELISA reader cannot read above A450 of 2.00-3.00 then reduce the incubation time.

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

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

9. Please Note: Due to plate reader differences, the high standard absorbance values may be out of range occasionally. If this occurs, absorbance values may be determined at 405 nm instead. If absorbance values exceed the high standard, the samples should be appropriately diluted with cTnI diluent and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero troponin-I value

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 well the same by adding the reagents in identical sequence. Plate readers measure absorbance vertically. Do not touch the bottom of the wells.