ELISA kits available from ADI:

Catalog#	ProdDescription
6240	Mouse Serum Amyloid A ELISA Kit
6250	Mouse Serum Haptoglobin ELISA Kit
6250-10	Dog Serum Haptoglobin ELISA Kit
6250-30	Rat Serum Haptoglobin ELISA Kit
6250-40	Pig Serum Haptoglobin ELISA Kit
6250-50	Cat Serum Haptoglobin ELISA Kit
6250-60	Bovine Serum Haptoglobin ELISA Kit
600-480-CTN	Rabbit Cardiac Tn-I ELISA kit for serum samples
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

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-6250-20

Horse Haptoglobin

ELISA KIT Cat. No. 6250-20

For Quantitative Determination of Haptoglobin in Horse Serum

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

Horse Haptoglobin ELISA KIT Cat. No. 6250-20

Kit Components, 96 tests	
Anti-Horse haptoglobin coated strip plate (8 wells x 12 strips), #6250-20-1	1 plate
Horse haptoglobin Reference Standard (100 ug/ml), lyophilized, *Reconstitute with dH_20 according to vial label, #6250-20-2*	1 vial
Anti-Horse haptoglobin-HRP Conjugate, 11 ml, #6250-20-3	1 bottle
Denaturing Buffer, 25 ml, #6250-20-4	1 bottle
Sample Diluent, (10X) 25 ml, #6250-20-SD	1 bottle
Wash Buffer (20X), 50 ml, #6250-20-WB	1 bottle
TMB Substrate, 11 ml, #6250-20-TMB	1 bottle
Stop solution, 11 ml, #6250-20-SS	1 bottle
Instruction Manual, #M-6250-20	1 manual

INTRODUCTION

The liver produces haptoglobin and secretes it into the blood. When red blood cells are destroyed, the hemoglobin is released. Haptoglobin binds to the released hemoglobin. Macrophages will then bring the haptoglobin-hemoglobin complex to the liver, where the haptoglobin and hemoglobin are separated and the iron is recycled. This process destroys the haptoglobin. When red blood cells are actively being destroyed, the rate of haptoglobin destruction by the liver will outpace the rate at which new haptoglobin is created, and the levels of haptoglobin in the blood will decrease.

Since the reticuloendothelial system will remove the haptoglobin-hemoglobin complex from the body, haptoglobin levels will be decreased in hemolytic anemias. In the process of binding hemoglobin, haptoglobin sequesters the iron within hemoglobin, preventing iron-utilizing bacteria from benefiting from hemolysis. It is theorized that, because of this, haptoglobin has evolved into an acute-phase protein. It has a protective influence on the hemolytic kidney.

Haptoglobin is an acute phase reactant protein. Its level increases during acute conditions such as infection, injury, tissue destruction, some cancers, burns, surgery, or trauma. Its level decreases during such conditions as chronic liver disease, hematoma, and hemolytic anemia.

ADI's Horse Haptoglobin ELISA provides is a rapid, specific and sensitive assay for measuring Horse Haptoglobin in serum or other biological solutions

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve on semi-log graph paper by plotting net absorbance values of standards against appropriate HAPTOGLOBIN concentrations. Read off the HAPTOGLOBIN concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples. If samples were diluted 1:20K then the values must be multiplied by 20,000 and results are expressed as ug/ml.

PERFORMANCE CHARACTERISTICS

Wash Procedure: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings

Detection Limit: The minimum HAPTOGLOBIN concentration detectable using this assay is below 3.0 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Horse HAPTOGLOBIN levels in serum may vary up to about 1.0 mg/ml. Each laboratory should establish testing ranges for the animal population being investigated.

Specificity: The antibodies used in this kit are specific for Horse haptoglobin and have shown no cross-reactivity with other serum proteins.

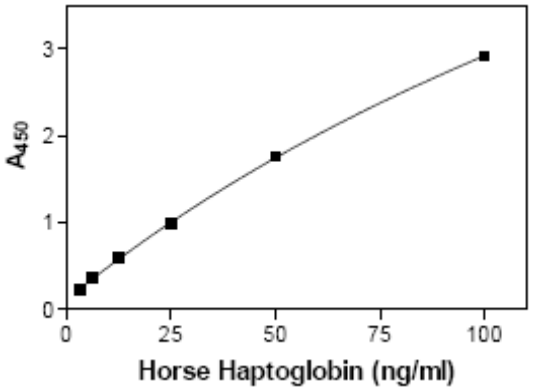
Species Crossreactivity: Cross-reactivity was tested with animal sera at dilutions of 1:100. **Rat, Mouse, Guinea pig, sheep and goat haptoglobin sera did not show good reactivity. Rabbit, bovine, human, and monkey sera were significantly positive.** Since we only tested the sera and not the purified haptoglobin, it is not possible ascertain the extent of crossreactivity. But the above information should provide some measure of anti-Horse haptoglobin reactivity with the other species.

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.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Negative Diluent Control**	0 ng/ml	0.068	
B1, B2	**Standard A**	3.13 ng/ml	0.233	
C1, C2	**Standard B**	6.25 ng/ml	0.365	
D1, D2	**Standard C**	12.5 ng/ml	0.590	
E1, E2	**Standard D**	25 ng/ml	0.988	
F1, F2	**Standard E**	50 ng/ml	1.756	
G1, G2	**Standard F**	100 ng/ml	2.919	
H1, H2	**Sample 1** 1:500 dilution		1.008	26 ng = **13.0** ug/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 Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Horse HAPTOGLOBIN ELISA kit is based on binding of Horse HAPTOGLOBIN 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 HAPTOGLOBIN 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 HAPTOGLOBIN 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 Horse HAPTOGLOBIN 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. **Sample Diluent (10X)** Dilute 1:10 using 10 ml diluent in 90ml water. Dilute only the required reagent. Store diluted solution at 2-8^{o} C.
2. **Wash Buffer (20X)**. Dilute the entire 50 ml with distilled or deionized water to 950 ml water (total volume 1000 ml). Store at room temperature for the entire use of the kit.
3. **Reference Standard** - See detailed preparation on page 3.

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

Studies indicate that α Horse Haptoglobin is present in normal Horse serum at a concentration of about 1.0 mg/ml. The normal values may changes with respect to strain, age, diet, and other factors. In order to obtain values within the range of the standard curve, we suggest that samples be diluted 100,000 fold using the following procedure for each sample to be tested:

i) Prepare 998 uL and 497.5 uL of sample diluent (1X) in separate tubes.
ii) Prepare 1:500 sample dilution by mixing 2.0 uL of sample into the tube containing the 998 uL of diluent (1X)
iii) Prepare 1:100,000 sample dilution by adding 2.5 uL of the 500 fold sample dilution to the 497.5 uL of diluent in second tube.

Repeat this procedure for each sample to be tested

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

1. Reconstitute the lyophilized reference standard with volume of distilled or deionized water indicated on the vial. This gives the concentration **of the reference standard as 100 ug/ml. Immediately aliquot and store** any unused reference standard at –20oC or below.

2. Prepare liquid standards using the following dilution scheme. Label 8 microcentrifuge tubes as 100, 50, 25, 12.5, 6.25, 3.13, and 0 ng/ml.

3. For standard F (100 ng/ml) pipet 10 uL of reference standard (100 ug/ml) in 190 uL of denaturing buffer, mix and incubate for 10 minutes at room temperature. Then, pipet 20 uL of the denatured standard into 980 uL of diluent (1X) and mix gently. Prepare the remaining standards as shown below.

Notes: When preparing the serial dilutions of the standards gently mix the standards for 5-10 seconds and then take aliquots to make further dilutions. Following the dilution scheme, you will have 500 uL of negative and all standards (B-F), G and 1000 ul of Std. A. You would need 200 uL of each standard (100 uL in duplicate).

Horse Haptoglobin Stds	Stock Volume	1X Diluent	Final Volume
Std F (100 ng/ml)	1000 uL	0	1000 uL
Std E (50 ng/ml)	500 uL of Std F	500 uL	1000 uL
Std D (25 ng/ml)	500 uL of Std E	500 uL	1000 uL
Std C (12.5 ng/ml)	500 uL of Std D	500 uL	1000 uL
Std B (6.25 ng/ml)	500 uL of Std C	500 uL	1000 uL
Std A (3.13 ng/ml)	500 uL of Std B	500 uL	1000 uL
Negative (0 ng/ml)	0	500 uL	500 uL

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

4. Pipet **100 uL standards and diluted samples** into appropriate wells. Mix gently, and incubate at room temperature (20-25oC) for **45 minutes** on an orbital shaker (100-150 rpm). If an automated shaker is not available, the plate can be mixed manually every few minutes.

5. Remove or aspirate the plate contents and **wash the wells 4-5 times** with 300 uL of 1x 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.

6. Pipet **100 uL of Ab-enzyme conjugate** into each well. Mix gently, and incubate for **30 minutes** at room temperature as in step 4.

7. **Wash the wells 4-5 times** as in step 5. Tap the plate over fresh paper towels to remove traces of liquid from the last washing step.

8. **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. 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 then reduce the incubation time.

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

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