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

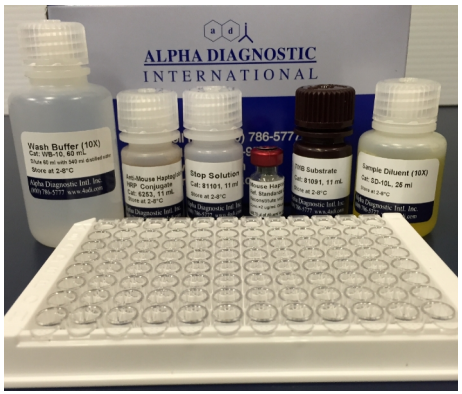
See Details at the web site or Contact ADI

Instruction Manual No. M-6250

Mouse Haptoglobin

ELISA KIT Cat. No. 6250

For Quantitative Determination of Haptoglobin in Mouse Serum



For In Vitro Research Use Only

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Mouse haptoglobin ELISA KIT Cat. No. 6250

Kit Components, 96 tests	Cat #
Anti-Mouse haptoglobin **coated strip plate** (8 wells x 12 strips)	6251
Mouse haptoglobin **Reference Standard** (2 ug/ml), lyophilized, *Reconstitute with dH_20 according to vial label*	6252
Anti-mouse haptoglobin-**HRP Conjugate**, 11 ml	6253
10x **Sample Diluent**, 25 ml	SD-10L
Wash Buffer (10x), 60 ml	WB-10
TMB Substrate, 11 ml	81091
Stop solution, 11 ml	81101
Instruction Manual	M-6250

NTRODUCTION

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.

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, hemolytic anemia.

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

DILUTION OF SAMPLES

Samples containing more than 125 ng/ml HAPTOGLOBIN 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 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

Detection Limit: The minimum HAPTOGLOBIN concentration detectable using this assay is below 1 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Mouse HAPTOGLOBIN levels in serum may vary from 0.1-2 mg/ml. Each laboratory should establish testing ranges for the animal population being investigated.

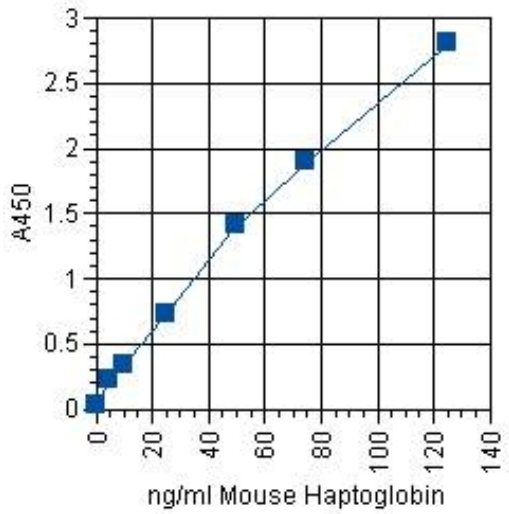
Specificity: The antibodies used in this kit are specific for mouse haptoglobin and have shown no cross-reactivity with other serum proteins.

Species Crossreactivity: Cross-reactivity was tested with animal sera at dilutions of 1:100. **Rat, Dog, G. pig, Horse, sheep and goat haptoglobin sera did not show good reactivity. Rabbit, bovine, goat, sheep, human, 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-mouse haptoglobin reactivity with the other species.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples	Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Negative Diluent Control 0 ng/ml**	0.086	
B1, B2	**Standard A** 1.95 ng/ml	0.258	
C1, C2	**Standard B** 3.9 ng/ml	0.404	
D1, D2	**Standard C** 7.8 ng/ml	0.726	
E1, E2	**Standard D** 15.6 ng/ml	1.241	
F1, F2	**Standard E** 31.2 ng/ml	1.98	
F1, F2	**Standard F** 62.5 ng/ml	3.086	
F1, F2	**Standard G** 125 ng/ml	3.94	
G1, G2	**Sample 1** 1:500 dilution	1.260	15.67 ng/ml = **7.8** 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

Mouse HAPTOGLOBIN ELISA kit is based on binding of Mouse 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 Mouse 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. **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° C for 3-4 days.

2. **Dilute Wash Buffer (10x stock)**. Dilute the entire 60 ml with distilled or deionized water to 540 ml water (total volume 600 ml). Store at room temperature for the entire use of the kit.

3. Standard preparation-it is provided as lyophilized stock. 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.

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 using the following dilution scheme. Label 8 microcentrifuge tubes as 125, 62.5, 31.2, 15.6, 7.8, 3.9, 1.95, and 0 ng/ml.

Mouse Haptoglobin Stds	Stock ug/ml) Volume	1X Diluent	Final Volume
Std G (125 ng/ml)	31.25 ul of **2 ug/ml** stock	468.8 uL	500 uL
Std F (62.5 ng/ml)	250 ul of Std G	250 uL	500 uL
Std E (31.2 ng/ml)	250 ul of Std F	250 uL	500 uL
Std D (15.6 ng/ml)	250 ul of Std E	250 uL	500 uL
Std C (7.8 ng/ml)	250 ul of Std D	250 uL	500 uL
Std B (3.9 ng/ml)	250 ul of Std C	250 uL	500 uL
Std A (1.95 ng/ml)	250 ul of Std B	250 uL	500 uL

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 above dilution scheme, you will have 250 ul of all standards (B-F) and 500 ul of Std. A. You would need 200 ul of each standards (100 ul in duplicate).

Diluting the mouse serum samples 1:20,000-1:40,000 (use 1x Sample Diluent) will bring most samples into the testing range. For those testing out of the range dilute accordingly. We recommend the following dilution scheme for the samples.

1. Take 10 ul of samples and 990 ul of 1x diluent and mix for 1:100 dilution
2. Take 10 ul of 1:100 dilution and 990 ul of 1x diluent for 1:10,000
3. take 200 ul of 1:10,000 dilution and 200 ul of diluent for 1:20,000

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

3. 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.

4. 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.

5. Pipette **100 ul of Ab-enzyme conjugate** into each well. Mix gently, and incubate for **30 minutes** at room temperature as in step 3.

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

6. **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 $\pm$ 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.

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.

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.