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
6250-20	Horse 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-6250-30

Rat Haptoglobin ELISA KIT

Cat. No. 6250-30, 96 Tests

For Quantitative Determination of Haptoglobin in Rat Serum, plasma or other biological fluids

For in-vitro research use only (RUO)

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Rat Haptoglobin ELISA KIT Cat. No. 6250-30

Kit Components, 96 tests	qty
Anti-Rat haptoglobin **coated strip plate** (8 wells x 12 strips), #6250-30-1P	1 plate
Rat haptoglobin **Reference Standard, lyophilized**, *Reconstitute with dH_20 according to vial label, #6250-30-2*	1 vial
Anti-Rat haptoglobin-**HRP Conjugate**, 11 ml, #6250-30-3	1 bottle
Sample Diluent (10X) 25 ml, #625030-SD	1 bottle
TMB Substrate, 11 ml, #625030-TMB	1 bottle
Wash Buffer (20X), 50 ml, #625030-WB	1 bottle
Stop solution, 11 ml, #625030-SS	1 bottle
Instruction Manual, #M-6250-30	1 manual

Intended Use:

ADI's Rat Haptoglobin ELISA is a sandwich immuonoassay for rapid, specific and sensitive measurements of Rat Haptoglobin in serum or other biological solutions. For in-vitro research use only (RUO).

INTRODUCTION

Haptoglobin (also known as Haptoglobin, alpha polypeptide antibody, Haptoglobin, beta polypeptide antibody, HP antibody, Hp2 alpha antibody, HP2 ALPHA2 antibody,HPA1S antibody, HPT antibody, MGC111141 antibody as Hp) is a protein that in humans is encoded by the HP gene. In blood plasma, haptoglobin binds free hemoglobin (Hb) released from erythrocytes with high affinity and thereby inhibits its oxidative activity. The haptoglobin-hemoglobin complex will then be removed by the reticulo-endothelial system (mostly the spleen).

This HP gene encodes a precursor that is processed to yield both alpha and beta chains, which subsequently combine as a tetramer to produce haptoglobin. Haptoglobin functions to bind free plasma hemoglobin, which allows degradative enzymes to gain access to the hemoglobin while at the same time preventing loss of iron through the kidneys and protecting the kidneys from damage by hemoglobin. Haptoglobin is produced mostly by hepatocytes but also by other tissues: e.g., skin, lung, and kidney. In addition, the haptoglobin gene is expressed in murine and human adipose tissue. Haptoglobin, in its simplest form, consists of two α- and two β-chains, connected by disulfide bridges. Hp exists in two allelic forms in the human population, so-called Hp1 and Hp2, the latter one having arisen due to the partial duplication of Hp1 gene. Three phenotypes of Hp, therefore, are found in humans: Hp1-1, Hp2-1, and Hp2-2. Hp of different phenotypes have been shown to bind hemoglobin with different affinities, with Hp2-2 being the weakest binder. The amino acid sequence of Hp1-1 consists of 406 aa, it has molecular weight of about 45 kDa.

Haptoglobin is an acute phase reactant protein produced by liver. 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.

CALCULATION OF RESULTS

Calculate the mean absorbance for each duplicate. Draw the standard curve 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 1:20,000 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 1.0 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Rat 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 Rat haptoglobin and have shown no cross-reactivity with other serum proteins.

Species Crossreactivity: Cross-reactivity of rat haptoglobin in other species is not tested.

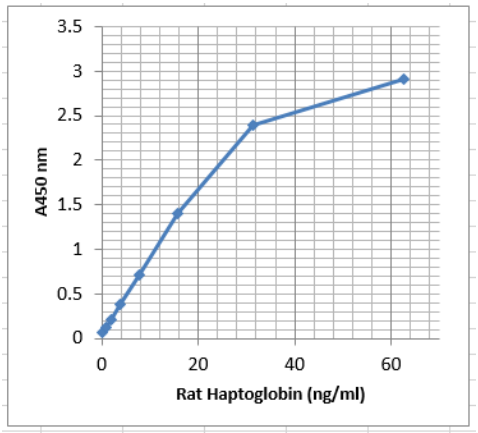
General References: van der Straten A. (1983) EMBO J., 2, 1003-1007; Yang F. (1983) PNAS USA, 80, 5875-5879; Maeda N. (1984) Nature, 309, 131-135; Kurosky A. (1980) PNAS USA, 77, 3388-3392; Kliffen M. (1995) Lab. Invest., 73, 267-272; Malchy B., and Dixon G.H. (1973) Can. J. Biochem., 51, 249-264; Dobryszycka W. (1997) Eur J Clin Chem Clin Biochem, 35, 647–54; Wassell J. (2000) Clin. Lab., 46, 547–52.; Trayhurn P., and Wood I.S. (2004) Br. J. Nutr., 92, 347–355; Sadrzadeh S.M., and Bozorgmehr J. (2004) Am. J. Clin. Pathol., 121, Suppl: S97–S104; Papp M. (2007) Dig. Dis. Sci., 52, 1279–1284.

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	0.062	
B1, B2	**Standard A**	0.98 ng/ml	0.125	
C1, C2	**Standard B**	1.95 ng/ml	0.214	
D1, D2	**Standard C**	3.9 ng/ml	0.382	
E1, E2	**Standard D**	7.8 ng/ml	0.710	
F1, F2	**Standard E**	15.6 ng/ml	1.395	
G1, G2	**Standard F**	31.2 ng/ml	2.393	
H1, H2	**Standard G**	62.5 ng/ml	2.911	
A3, A4	**Sample 1**		1.2918	15.5 ng

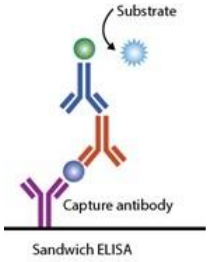
NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.



*6-ADI_ELISA

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

PRINCIPLE OF THE TEST



Rat HAPTOGLOBIN ELISA kit is based on binding of Rat 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 Rat 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.

Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). 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. http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf.

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. **Reference Standard** - See detailed preparation on page 3.
3. **Dilute Wash Buffer (20x stock)**. Dilute the entire **50 ml with 950 ml of distilled** or deionized water (total volume 1000 ml). Store at room temperature for the entire use of the kit. It can be stored at 4oC for long term storage.

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 Rat Haptoglobin is present in normal Rat 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 100,000-200,000 fold using the following multistep procedure for each sample to be tested:

Sample	1X diluent	Total volume	Sample Dilution
5 ul	995 ul	1000 ul	1:200
5 ul of 1:200	995 ul	1000 ul	1:40,000
100 ul of 1:40,000	150 ul	250 ul	1:100,000

Each sample dilution should be mixed for few seconds before proceeding for the next dilution. The above scheme is multistep process and minimize large dilution and errors. Additional sample dilution of 1:200,000 or more can be prepared from 1:40,000. Keep all sample dilutions at 4oC or freeze for long term use.

Preparation of Working standards

1. Reconstitute the lyophilized reference standard with volume of distilled or deionized water indicated on the vial. Light mix the content and let it mix for 15-30 mins at room temperature with gently shaking on an orbital shaker or end to end mixer. This gives the concentration **of the reference standard as 2000 ng/ml. Label this as Rat Haptoglobin stock standard (2000 ng/ml). Immediately aliquot in 100 ul and store** any unused reference standard at –20oC or below. Use 1 vial for making required standards of 62.5-1.98 ng/ml as given below. Do not use or store this vial and use fresh stock when needed.

Rat Haptoglobin Stds	Stock Volume	1X Diluent	Final Volume
Std G (62.5 ng/ml)	15.62 uL of Std H	484.3 uL	500 uL
Std F (31.2 ng/ml)	250 uL of Std G	250 uL	500 uL
Std E (15.6 ng/ml)	250 uL of Std F	250 uL	500 uL
Std D (7.8 ng/ml)	250 uL of Std E	250 uL	500 uL
Std C (3.9 ng/ml)	250 uL of Std D	250 uL	500 uL
Std B (1.95 ng/ml)	250 uL of Std C	250 uL	500 uL
Std A (0.98 ng/ml)	250 uL of Std B	250 uL	500 uL
Negative (0 ng/ml)	0	250 uL	250 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 dilution scheme, you will have 250 uL of negative and all standards (B-F),except 500 ul of Std. A. You would need 200 uL of each standard (100 uL in duplicate).

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

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

2. Pipet **100 uL working standards and diluted samples** into appropriate wells in duplciate. 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.

3. Remove or aspirate the plate contents and **wash the wells 4-5 times** with 300 uL of distilled or deionized water using an automated washer. If washing manually then dump the plate contents and tap over paper towels, add water, 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.