ELISA kits available from ADI:

Catalog#	ProdDescription
600-320-LFP	Mouse Liver Fatty Acid Binding Protein ELISA Kit
600-400-CTN	Dog Cardiac Troponin 1 (Tn-I) ELISA Kit
600-410-CTN	Human Cardiac Troponin 1 (Tn-I) ELISA Kit
600-420-CTN	Monkey Cardiac Troponin 1 (Tn-I) ELISA Kit
600-430-MTN	Monkey Skeletal Muscle Troponin1 (Tn-I) ELISA Kit
600-440-CTN	Mouse Cardiac Tn-I ELISA kit for plasma samples
600-450-CTN	Mouse Cardiac Troponin 1 (Tn-I) ELISA Kit
600-470-CTN	Pig Cardiac Troponin 1 (Tn-I) 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
600-620-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-600610-HMY

Human Myoglobin (HMY)

ELISA KIT Cat. # 600-610-HMY

For Quantitative Determination of Myoglobin in Human Serum and Plasma

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

Human Myoglobin (HMY) ELISA KIT
Cat. No. 600-610-HMY

Kit Components, 96 tests	
Anti-Myoglobin coated strip plate (8 wells x 12 strips), #600610-1	1 plate
HMY Reference Standard Set, 1.0 ml each, #600610-2	5 vial
Anti-HMY-HRP Conjugate, 22 ml, #600610-3	1 bottle
Sample Diluent, 25 ml, #600610-4	1 bottle
TMB Substrate, 11 ml, #600610-TMB	1 bottle
Stop solution, 11 ml, #600610-SS	1 bottle
Instruction Manual, # M-600610	1 manual

INTRODUCTION

Myoglobin is a single-chain globular protein with a molecular weight of 16,700 daltons. It contains a heme prosthetic group in the center and is the primary oxygen-carrying pigment of muscle tissues.

Myoglobin is found in both cardiac and skeletal muscle. Following myocardial necrosis associated with myocardial infarction (MI), myoglobin is one of the first markers to rise above normal levels. Studies with human subjects have shown that myoglobin increases measurably above baseline within 2-4 hours post-infarct, peaking at 9-12 hours, and returning to baseline within 24-36 hours. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin may used as a marker for MI. Similarly, in the absence of cardiac damage, myoglobin may be used as a marker of skeletal muscle injury.

Myoglobin is released from the muscle tissue following excessive exertion (RHABDOMYOLYSIS) or trauma such as torn muscle fibers. The quantitation of myoglobin in serum and urine is of clinical importance for the differentiation of myocardial infarction from degenerative cardiac disorders as well as for the detection of traumatic and atraumatic rhabdomyolysis, followed frequently by acute kidney failure.

ADI's Human Myoglobin (HMY) ELISA provides a rapid, specific and sensitive assay for measuring HMY in serum or other biological fluids.

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 HMY concentrations. Read off the HMY 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.

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.

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 HMY concentration detectable using this assay is below 5 ng/ml. The detection limit is defined as the value deviating by 2 SD from the zero standard.

Expected Values: Each laboratory should establish testing ranges for the animal population being investigated.

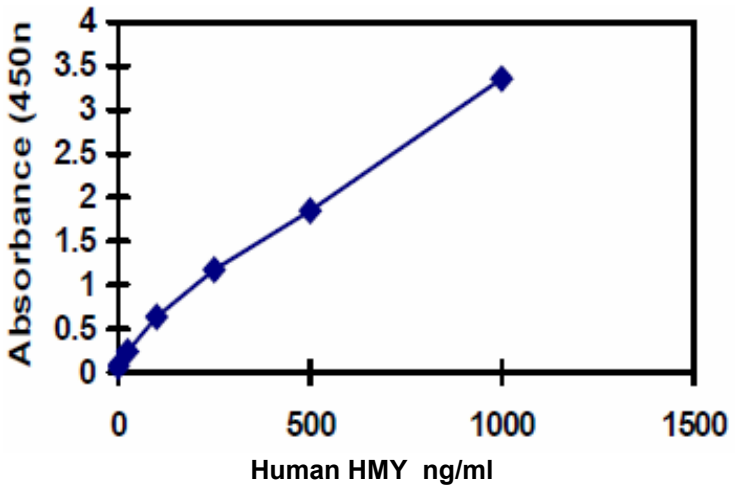
Specificity: The antibodies used in this kit are specific for Human HMY and have shown no cross-reactivity with other proteins.

Species Crossreactivity: Cross-reactivity of Human HMY ELISA kit with other animals has not been tested. ADI has myoglobin ELISA kits for dog, mouse, monkey, pig, rabbit and rat.

WORKSHEET OF TYPICAL ASSAY

Wells	Stds/samples		Mean $A_{450\ nm}$	Calculated Concn
A1, A2	**Sample Diluent**	0 ng/ml	0.071	
B1, B2	**Standard A**	25 ng/ml	0.235	
C1, C2	**Standard B**	100 ng/ml	0.632	
D1, D2	**Standard C**	250 ng/ml	1.169	
E1, E2	**Standard D**	500 ng/ml	1.845	
F1, F2	**Standard E**	1000 ng/ml	3.357	
G1, G2	**Sample 1**		1.118	245 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 Curve (do not use this for calculating sample values)

PRINCIPLE OF THE TEST

Human HMY ELISA kit is based on binding of Human HMY 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 HMY 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 HMY 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 Human Troponin-I ELISA Kit is for research use only.

Caution: 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. Plasma samples may be collected in tubes containing EDTA. If sera cannot be immediately assayed, store frozen for up to six months. Avoid repeated freezing and thawing of samples. **Cell or tissues extract samples have not been optimized.**

REAGENT PREPARATION

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.

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.

DILUTION OF SAMPLES

Serum or plasma samples should be diluted ten-fold before being tested. For example, use 20 uL of sample and dilute it with 180 uL of sample diluent. High value samples (greater than 1000 ng/ml) should be further diluted ten-fold with sample diluent prior to reassaying.

TEST PROCEDURE

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

1. The **concentration of the reference standards are , 25, 100, 250, 500, and 1000 ug/ml.** Do not dilute the standards as they have already been pre-diluted 10-fold.

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

2. Pipet **20 ul standards (undiluted), controls (diluted) and samples (diluted)** into appropriate wells.

3. Pipette **200 ul of anti-HMY-HRP conjugate** into each well. Mix well for 30 seconds, and incubate at room temperature (20-25oC) for **45 minutes.**

4. Remove or aspirate the plate contents and **wash the wells 5-6 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. **Do not use tap water for washing.**

5. 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 2.00-3.00 then reduce the incubation time.

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

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

8. 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 and redetermined. Samples with absorbance values below those of the lowest standard should be assigned a zero HMY 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.

LIMITATIONS OF THE ASSAY

Samples may contain human anti-mouse antibodies (HAMA), which are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interferences from HAMA-containing specimens. Nevertheless, complete elimination of the interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.