ELISA kits available from ADI:

Catalog#	ProdDescription
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
600-600-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
600-650-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-600660-RMY

Rat Myoglobin (RMY)

ELISA KIT Cat. # 600-660-RMY

For Quantitative Determination of Myoglobin in Rat Serum/Plasma/Urine

ALPHA DIAGNOSTIC INTERNATIONAL

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

Rat Myoglobin (RMY) ELISA KIT
Cat. No. 600-660-RMY, 96 Tests

Kit Components, 96 tests	Cat #
Anti-Myoglobin coated strip plate (8 wells x 12 strips), #600661	1 plate
RMY Reference Standard stock 50 ug/ml (500x), #600662, diluted to prepare other standards (lot specific concn of the stock is provide on the vial) Store at -20oC ,	1 vial
Anti-RMY IgG-HRP Conjugate, 11 ml, #600663	1 bottle
Std/Sample Diluent, 12 ml, #600664	1 bottle
Wash Buffer (20x), 50 ml, #600660WB	1 bottle
TMB Substrate, 11 ml, #600660TMB	1 bottle
Stop solution, 11 ml, #600660SS	1 bottle
Instruction Manual, # M-600660-RMY	I manual

INTRODUCTION

Myoglobin is a heme protein 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. Blood, Hemoglobin and Myoglobin all give a positive result to occult blood with a dipstick test.

Myoglobin in the urine (Myoglobinuria) is characterized by brownish red urine. The presence of Myoglobin in the urine indicates the need for a thorough muscle check, and a blood test for muscle enzymes to rule out muscle disease. Myoglobin in the urine may also adversely affect the kidneys (toxicity).

ADI's Rat Myoglobin (RMY) ELISA provides a rapid, specific and sensitive assay for measuring RMY 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 RMY concentrations. Read off the RMY concentrations of the control and patient samples. Multiply the values by the dilution factor of the samples.

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 RMY concentration detectable using this assay is below 0.5 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 Rat RMY and have shown no cross-reactivity with other proteins.

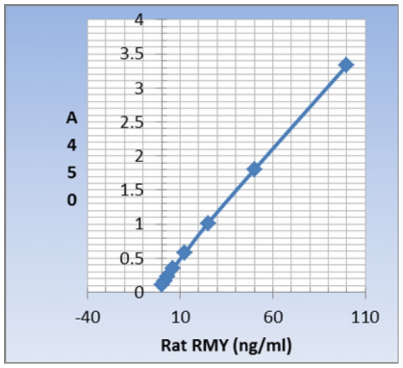
Species Crossreactivity: Cross-reactivity of Rat RMY ELISA kit with other animals has not been tested. ADI has myoglobin ELISA kits for dog, human, monkey, mouse, rabbit and Pig.

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	**Diluent (** 0 ng/ml)	0.110	
B1, B2	**Standard A (**1.563 ng/ml)	0.158	
C1, C2	**Standard B (**3.125 ng/ml)	0.223	
D1, D2	**Standard C (**6.25 ng/ml)	0.356	
E1, E2	**Standard D (**12.5 ng/ml)	0.577	
F1, F2	**Standard E (**25 ng/ml)	1.015	
G1, G2	**Standard F (**50 ng/ml)	1.997	
H1, H2	**Standard G (**100 ng/ml)	3.333	
A3, A4	**Sample 1**		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.



3-ADI-graph

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

PRINCIPLE OF THE TEST

Rat RMY ELISA kit is based on binding of Rat RMY 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 RMY 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 RMY 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 RMY 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 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

1. **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.

2. **Reference Standard** is provided as liquid stock. Dilute it further to make standards as on page 3.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8oC until the expiration date printed on the label. The RMY reference standard should be stored at -20oC.

DILUTION OF SAMPLES

Serum or plasma samples can be tested undiluted. High value samples should be diluted with sample diluent prior to assay. Urine samples may show a matrix effect. So, all urine samples within an assay run should be similarly diluted prior to the assay.

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

1. The **concentration of the reference standard is 50 ug/ml. Immediately aliquot and store** any unused reference standard at –20oC or below. Prepare other refs. standards fresh prior to the assay and do not store for more than 30 min-1 hr.

2. Prepare liquid standards using the following dilution scheme. Label 8 microcentrifuge tubes as 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0 ng/ml.

3. For **standard G (100 ng/ml**) pipette 998 uL RMY diluent and add 2.0 uL of RMY reference standard (or as described on the RMY reference standard vial label; the stock concentration is lot specific and provided on the vial) and mix gently. Prepare the remaining standards as shown below.

RMY Stds	Stock Volume	Std. diluent	Final Volume
Std G (100 ng/ml)	1000 uL	0	1000 uL
Std F (50 ng/ml)	100 uL of Std G	100 uL	200 uL
Std E (25 ng/ml)	100 uL of Std F	100 uL	200 uL
Std D (12.5 ng/ml)	100 uL of Std E	100 uL	200 uL
Std C (6.25 ng/ml)	100 uL of Std D	100 uL	200 uL
Std B (3.125 ng/ml)	100 uL of Std C	100 uL	200 uL
Std A (1.56 ng/ml)	100 uL of Std B	100 uL	200 uL
Diluent (0 ng/ml)	0	100 uL	100 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 100 uL of negative and all standards (B-F), 900 uL of std. G and 200 ul of Std. A. You would need 40 uL of each standard (20 uL in duplicate).

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

4. Pipette **100 ul of anti-RMY-HRP conjugate** into each well.

5. Pipet **20 ul standards and samples** into appropriate wells. Mix gently, and incubate at room temperature (20-25oC) for **60 minutes on an orbital shaker (100-150 rpm).** If an automated shaker is not available, the plate can be mixed manually every few minutes.

6. Remove or aspirate the plate contents and **wash the wells 5-6 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.

7. **Add 100 ul of TMB Substrate** into each well. Mix gently. Cover the plate and incubate for **20 minutes** at room temperature **on an orbital shaker (100-150 rpm)**. 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.

8. Stop the reaction by adding **100 ul of stop solution** to all wells. Mix gently for 30 seconds. Blue color turns yellow.

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

10. 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 RMY value