

KAMIYA BIOMEDICAL COMPANY

Rat Myoglobin ELISA

**For the quantitative determination of myoglobin
in rat biological samples**

Cat. No. KT-420

For research use only.

PRODUCT INFORMATION

**Rat Myoglobin ELISA
Cat. No. KT-420**

INTENDED USE

The Rat Myoglobin ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of myoglobin in rat biological samples. For research use only.

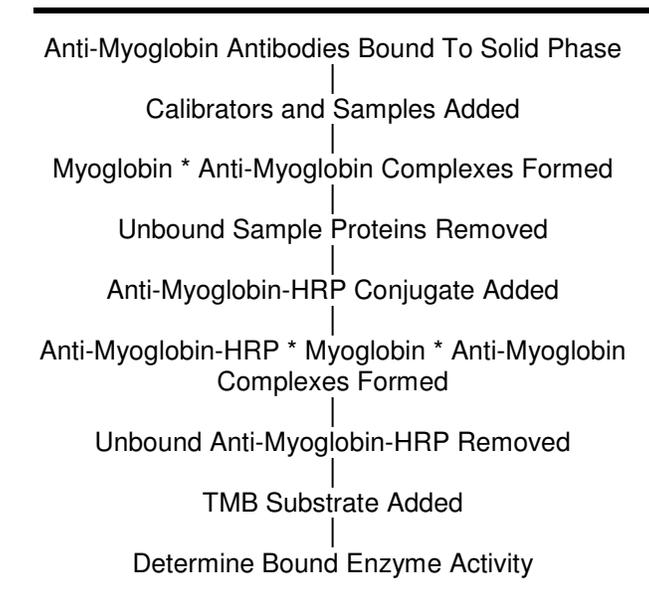
INTRODUCTION

Myoglobin is a single-chain globular protein containing a heme group in the center. Myoglobin is found in both cardiac and skeletal muscle tissues. It is released from damaged muscle tissues and therefore makes it a sensitive marker for muscle injury.

PRINCIPLE

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the myoglobin present in the sample reacts with the anti-myoglobin antibodies which have been adsorbed to the surface of polystyrene microtiter wells. After the removal of unbound proteins by washing, anti-myoglobin antibodies conjugated with horseradish peroxidase (HRP) are added. This HRP-conjugated antibody forms a complex with the previously bound myoglobin. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme is proportional to the concentration of myoglobin in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of myoglobin in the test sample. The quantity of myoglobin in the test sample can be interpolated from the calibration curve constructed from the calibrators, and corrected for sample dilution.

Figure 1.



COMPONENTS

1. Diluent Concentrate
One bottle containing 50 mL of a 5X concentrated diluent running buffer.
2. Wash Solution Concentrate
One bottle containing 50 mL of a 20X concentrated wash solution.

3. Enzyme-Antibody Conjugate Concentrate
One vial containing 150 μ L of a 100X concentrated affinity-purified anti-rat myoglobin antibody conjugated with HRP in stabilizing buffer.
4. TMB Substrate Solution
One vial containing 12 mL of TMB and hydrogen peroxide in citric acid buffer at pH 3.3.
5. Stop Solution
One vial containing 12 mL of 0.3 M sulfuric acid. **WARNING:** Avoid contact with skin.
6. Microtiter Plate
Twelve removable eight-well micro strips in well holder frame. Wells are coated with affinity-purified anti-rat myoglobin.
7. Rat Myoglobin Calibrator
One vial containing 1.0 mL of a Rat Myoglobin Calibrator.
8. Positive Control
One vial containing 50 μ L of serum with 0.1% sodium azide. See the Control Certificate for the concentration.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 μ L to 200 μ L) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Distilled or de-ionized H₂O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer
- Vortex mixer

PRECAUTIONS

1. Read the instructions carefully before beginning the assay.
2. This kit is for research use only.
3. Great care has been taken to ensure the quality and reliability of this product. However, it is possible that in certain cases, unusual results may be obtained due to high levels of interfering factors.
4. Preservatives
 - Positive Control contains 0.1% sodium azide.
5. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.
6. Azide and thimerosal at concentrations higher than 0.1% inhibit the enzyme reaction.
7. Other precautions:
 - Do not interchange kit components from different lots.
 - Do not use kit components beyond the expiration date.
 - Protect reagents from direct sunlight.
 - Do not pipette by mouth.
 - Do not eat, drink, smoke or apply cosmetics where reagents are used.
 - Avoid all contact with the reagents by using gloves.
 - Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

REAGENT PREPARATION

1. Diluent Concentrate
The Diluent solution supplied is a 5X concentrate and must be diluted 1:5 with distilled or de-ionized water.
2. Wash Solution Concentrate
The Wash Solution supplied is a 20X concentrate and must be diluted 1:20 with distilled or de-ionized water. Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. Enzyme-Antibody Conjugate Concentrate

Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 μL Enzyme-Antibody Conjugate to 990 μL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. TMB Substrate Solution
Ready to use as supplied.5. Stop Solution
Ready to use as supplied.

6. Microtiter Plate

Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

7. Rat Myoglobin Calibrator

The Rat Myoglobin Calibrator should be aliquoted out and stored frozen. The calibrator is now at a concentration of 2.40 $\mu\text{g}/\text{mL}$. **Rat Myoglobin calibrators need to be prepared immediately prior to use (see chart below).** Mix well between each step. Avoid foaming.

Calibrator	Concentration (ng/mL)	Calibrator Volume added to 1X Diluent	Volume of 1X Diluent
7	200	60 μL Rat Myoglobin Calibrator	660 μL
6	100	0.3 mL Calibrator 7	0.3 mL
5	50	0.3 mL Calibrator 6	0.3 mL
4	25	0.3 mL Calibrator 5	0.3 mL
3	12.5	0.3 mL Calibrator 4	0.3 mL
2	6.25	0.3 mL Calibrator 3	0.3 mL
1	3.125	0.3 mL Calibrator 2	0.3 mL
0	0		0.5 mL

8. Positive Control

The concentration and recommended dilution are provided on the Control Certificate. Before use, briefly centrifuge the Positive Control to allow all of the liquid to collect in the bottom of the vial.

STORAGE AND STABILITY

1. Complete Kit

The expiration date for the kit is stated on the outer label. The recommended storage temperature is 4°C. **Note: See long term storage recommendations below for the Rat Myoglobin Calibrator and Positive Control.**

2. Diluent

The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4°C.

3. Wash Solution

The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (RT, 16-25°C) or at 4°C.

4. Enzyme-Antibody Conjugate

Undiluted anti-myoglobin-HRP conjugate should be stored at 4°C and diluted immediately prior to use. The working conjugate solution is stable for up to 8 hours.

5. TMB Substrate Solution

The TMB Substrate Solution should be stored at 4°C and is stable until the expiration date.

6. Stop Solution

The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. Microtiter Plate

Anti-rat myoglobin coated wells are stable until the expiration date and should be stored at 4°C in the sealed foil pouch with a desiccant pack.

8. Rat Myoglobin Calibrator
Long Term Storage: Upon receipt, aliquot the calibrator and store them frozen. They will be stable until expiration date. Short Term Storage: The calibrator is stable for up to 14 days at 4°C. The working calibrator solutions should be prepared immediately prior to use and are stable for up to 8 hours.
9. Positive Control
For storage longer than 7 days keep frozen until the expiration date. Storage less than 7 days can be at 4°C. Avoid multiple freeze/thaw cycles.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freezing/thawing.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Samples

The assay for quantification of Myoglobin in samples requires that each test sample be diluted before use. For a single step determination a 1:2 dilution is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

To prepare a 1:2 dilution of sample, transfer 150 µL of sample to 150 µL of 1X Diluent. This gives you a 1:2 dilution. Mix thoroughly.

Procedure

1. Bring all reagents to RT before use.
2. Pipette 100 µL of
 - Calibrator 0 (0.0 ng/mL) in duplicate
 - Calibrator 1 (3.125 ng/mL) in duplicate
 - Calibrator 2 (6.25 ng/mL) in duplicate
 - Calibrator 3 (12.5 ng/mL) in duplicate
 - Calibrator 4 (25 ng/mL) in duplicate
 - Calibrator 5 (50 ng/mL) in duplicate
 - Calibrator 6 (100 ng/mL) in duplicate
 - Calibrator 7 (200 ng/mL) in duplicate
3. Pipette 100 µL of Positive Control (in duplicate) into pre designated wells.
4. Pipette 100 µL of sample (in duplicate) into pre designated wells.
5. Incubate the Microtiter Plate at 22°C (RT) for sixty (60 ± 2) minutes. Keep plate covered and level during incubation.
6. Following incubation, aspirate the contents of the wells.
7. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.

8. Pipette 100 μ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at 22°C (RT) for fifteen (15 \pm 2) minutes. Keep plate covered in the dark and level during incubation.
9. Wash and blot the wells as described in Steps 6 and 7.
10. Pipette 100 μ L of TMB Substrate Solution into each well.
11. Incubate in the dark at RT for precisely ten (10) minutes.
12. After ten (10) minutes, add 100 μ L of Stop Solution to each well.
13. Determine the absorbance at 450 nm of the contents of each well. Zero the plate reader to air.

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.
2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.
3. Interpolate test sample values from calibration curve. Correct for sample dilution factor to arrive at the myoglobin concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific myoglobin require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.

FOR RESEARCH USE ONLY

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