
DOG MYOGLOBIN ELISA

Life Diagnostics, Inc., Catalog Number: 2110-4

Enzyme Immunoassay for the Quantitative Determination of Myoglobin in Dog Serum or Plasma

STORAGE

Store the Myoglobin Standard at -20°C
Store remainder of kit at 2 to 8°C

INTRODUCTION

Myoglobin is a heme protein found in both cardiac and skeletal muscle. Following muscle damage myoglobin is one of the first markers to rise above normal levels in serum or plasma. Studies at Life Diagnostics, Inc., indicate that myoglobin increases measurably above baseline within 1-4 hours after cardiac damage in several species. In the absence of skeletal muscle trauma or other factors associated with a noncardiac related increase in circulating myoglobin, myoglobin levels can be used to detect and monitor cardiac damage.

PRINCIPLE OF THE TEST

The Myoglobin ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay uses a monoclonal antibody directed against a distinct antigenic determinant on the myoglobin molecule for solid phase immobilization (on the microtiter wells). A polyclonal anti-myoglobin antibody conjugated to horseradish peroxidase is used in the antibody-enzyme conjugate solution. The test sample is allowed to react simultaneously with the two antibodies, resulting in the myoglobin molecules being sandwiched between the solid phase and enzyme-linked antibodies. After a 60 minute incubation at room temperature, the wells are washed to remove unbound HRP-labeled antibodies. TMB (Tetramethyl-benzidine) Reagent is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is stopped by addition of Stop Solution, changing the color to yellow. The concentration of myoglobin is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS

Materials provided with the kit:

- Murine Monoclonal Anti-Myoglobin-coated microtiter wells, 96 wells
- Dog Cardiac Myoglobin Standard: 100 µg/ml
- Sample Diluent, 25 ml
- Enzyme Conjugate Reagent, 22 ml
- 10x Wash Solution, 60 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes
- Disposable pipette tips
- Distilled water
- Vortex mixer or equivalent
- Absorbent paper or paper towels
- Graph paper (PC graphing software is optional but recommended)
- Plate shaker
- Microtiter plate reader

SAMPLE COLLECTION AND PREPARATION

Serum or plasma should be collected using standard techniques. Remove serum or plasma from the coagulated or packed cells within 60 minutes after collection. Plasma samples may be collected into tubes containing EDTA. Samples that cannot be assayed within 3 hours of collection should be frozen at -20°C or lower.

Samples should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter should be centrifuged prior to use.

INSTRUMENTATION

A microtiter plate reader with an optical density range of 0-4 OD at 450 nm wavelength is acceptable for use in absorbance measurement.

WASH SOLUTION PREPARATION

The wash solution is provided as a 10x stock. Prior to use dilute the contents of the bottle (60 ml) with 540 ml of distilled or deionized water.

STANDARD PREPARATION

1. All reagents except the 100 µg/ml myoglobin stock vial should be brought to room temperature (18-25°C) before use.
2. Remove the 100 µg/ml myoglobin stock vial from the -20°C freezer and store it on ice temporarily. Ensure that the contents of the vial are mixed by brief vortexing. Flick or briefly centrifuge the solution to the bottom of the vial before use.
3. Label 8 polypropylene tubes as 500, 250, 125, 62.5, 31.25, 15.63, 7.81 and 0 ng/ml.
4. Pipette 398 µl of diluent into the tube labeled 500 ng/ml
5. Pipette 100 µl of diluent into the tubes labeled 250, 125, 62.5, 31.25, 15.63, 7.81 and 0 ng/ml.
6. Dilute 2.0 µl of the 100 µg/ml myoglobin stock into the 398 µl of diluent in the tube labeled 500 ng/ml. This provides a 500 ng/ml solution of myoglobin. Return the unused 100 µg/ml myoglobin standard to the -20°C freezer.

7. Prepare a 250 ng/ml stock by diluting and mixing 100 μ l of the 500 ng/ml stock with 100 μ l of diluent in the tube labeled 250 ng/ml. Similarly prepare the 125, 62.5, 31.25, 15.63, and 7.81 ng/ml stocks by serial dilution.

ASSAY PROCEDURE

1. It may be necessary to dilute serum or plasma prior to assay. The dilution factor should be determined empirically and samples should be diluted with the assay diluent. We recommend that all samples be similarly diluted.
2. Secure the desired number of coated wells in the holder.
3. Dispense 20 μ l of myoglobin standards and samples (in duplicate) into the appropriate wells.
4. Dispense 200 μ l of Enzyme Conjugate Reagent into each well.
5. Incubate at room temperature (18-25°C) on a plate shaker for one hour. Mix Gently
6. Remove the incubation mixture by flicking plate contents into a waste container.
7. Wash and empty the microtiter wells 5-6 times with wash solution. This may be performed using either a plate washer (400 μ l/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
8. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash solution.
9. Dispense 100 μ l of TMB Reagent solution into each well.
10. Incubate on a plate shaker at room temperature for 20 minutes. Mix Gently
11. Stop the reaction by adding 100 μ l of Stop Solution to each well.
12. Gently mix 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.
13. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

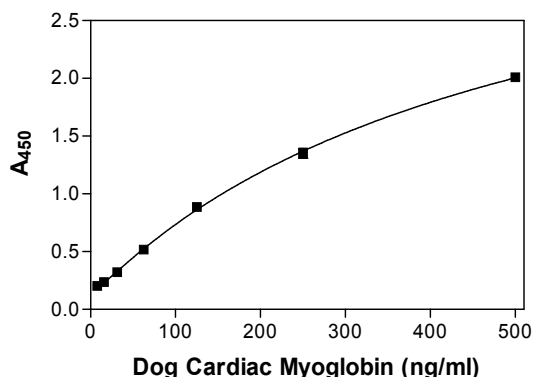
CALCULATION OF RESULTS

1. Calculate the mean absorbance value for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance values on the vertical or Y axis, and concentrations on the horizontal or X axis.
3. Use the mean absorbance values for each sample to determine the corresponding concentration of myoglobin in ng/ml from the standard curve.
4. If the samples were diluted prior to assay, multiply the derived value by the appropriate dilution factor.
5. Graphing software, if available, should be used.

TYPICAL STANDARD CURVE

Results of a typical standard run with optical density readings at 450 nm shown in the Y axis against myoglobin concentrations shown in the X axis are illustrated below. This standard curve is for illustrative purposes only, and should not be used to calculate unknowns. A standard curve should be run for each assay.

Dog Myoglobin Standard Curve



Myoglobin (ng/ml)	Absorbance (450 nm)
0	0.086
7.81	0.203
15.63	0.237
31.25	0.322
62.5	0.517
125	0.886
250	1.351
500	2.09

LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the instructions.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.