Mouse Myoglobin ELISA Kit

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Hinweis/Note:
Der Packungsbeileger dient nur als erste Information.
Der relevante Packungsbeileger liegt der Ware bei.

The datasheet is only a first information.
The relevant datasheet is included with the product.

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.

Symbol Key

Consult instructions for use.

Assay Summary

Add 50 µl of Standard/ Sample per well.
Incubate 2 hours.

⇩

Wash, then add
50 µl of Biotinylated Antibody per well.
Incubate 1 hour.

⇩

Wash, then add
50 µl of SP Conjugate per well.
Incubate 30 minutes.

⇩

Wash, then add
50 µl of Chromogen Substrate per well.
Incubate 20 minutes.

⇩

Add 50 µl of Stop Solution per well.
Read at 450 nm immediately.

Assay Template

	A	B	C	D	E	F	G	H
1								
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AssayMax Mouse Myoglobin ELISA Kit

Catalog No. EMM8001-1
Sample Insert/Reference Only

Introduction

Myoglobin is a heme-containing globular protein that is expressed in skeletal and cardiac muscles (1, 2). Human myoglobin consists of a single polypeptide chain of about 154 amino acid residues with a molecular weight of 17.6 kDa. It contributes oxygen storage and diffusion and functions as a radical scavenger and prevents hypoxia. In the cardiovascular system, myoglobin protein is abundantly expressed in the cytoplasm of cardiomyocytes and to a much lesser extent vascular smooth muscle (3). It has nitric oxide dioxygenation activity which serves as a nitrite reductase and intracellular catalyst (4). In rhabdomyolysis, the release of myoglobin exceeds the binding capacity of haptoglobin and causes renal failure. Blood myoglobin could be a valuable early predictor and marker of rhabdomyolysis (5). Myoglobin is induced by hypoxia in breast cancer cell lines and might have tumor-suppressive functions (6).

Principle of the Assay

The AssayMax Mouse Myoglobin ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for detection of mouse myoglobin in plasma, serum, urine, tissue extracts, and cell culture samples. This assay employs a quantitative sandwich enzyme immunoassay technique which measures mouse myoglobin in less than 4 hours. A polyclonal antibody specific for mouse myoglobin has been pre-coated onto a 96-well microplate with removable strips. Myoglobin in standards and samples is sandwiched by the immobilized antibody and a biotinylated polyclonal antibody specific for mouse myoglobin, which is recognized by a streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.

Caution and Warning

- **Prepare all reagents (working diluent buffer, wash buffer, standard, biotinylated antibody, and SP conjugate) as instructed, prior to running the assay.**

- **Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this protocol. However, the user should determine the optimal dilution factor.**
- **Spin down the SP conjugate vial and the biotinylated antibody vial before opening and using contents.**
- This kit is for research use only.
- The kit should not be used beyond the expiration date.
- The Stop Solution is an acidic solution.

Reagents

- **Mouse Myoglobin Microplate:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against mouse myoglobin.
- **Sealing Tapes:** Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Mouse Myoglobin Standard:** Mouse myoglobin in a buffered protein base (960 ng, lyophilized).
- **Biotinylated Mouse Myoglobin Antibody (50x):** A 50-fold concentrated biotinylated polyclonal antibody against mouse myoglobin (140 µl).
- **MIX Diluent Concentrate (10x):** A 10-fold concentrated buffered protein base (30 ml).
- **Wash Buffer Concentrate (20x)**: A 20-fold concentrated buffered surfactant (30 ml, 2 bottles).
- **Streptavidin-Peroxidase Conjugate (SP Conjugate):** A 100-fold concentrate (80 µl).
- **Chromogen Substrate**: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml).
- **Stop Solution**: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).

Storage Condition

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store SP Conjugate and Biotinylated Antibody at -20°C.
- Store Microplate, Diluent Concentrate (10x), Wash Buffer, Stop Solution, and Chromogen Substrate at 2-8°C.
- Unused microplate wells may be returned to the foil pouch with the desiccant packs and resealed. May be stored for up to 30 days in a vacuum desiccator.
- Diluent (1x) may be stored for up to 30 days at 2-8°C.
- Store Standard at 2-8°C before reconstituting with Diluent and at -20°C after reconstituting with Diluent.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 450 nm.
- Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel).
- Deionized or distilled reagent grade water.

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and assay. If necessary, dilute samples within the range of 1x – 5x with MIX Diluent. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).
- **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes. Remove serum and assay. If necessary, dilute samples within the range of 1x – 5x with MIX Diluent. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes and assay. If necessary, dilute samples within the range of 1x – 5x with MIX Diluent. The user should determine the optimal dilution factor. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 0.1 M phosphate-buffered saline (pH7.4) containing 1% Triton X-100 and centrifuge at 14000 x *g* for 20 minutes. Collect the supernatant and measure the protein concentration. Freeze remaining extract at -20°C or below.
- **Cell Culture Supernatants:** Centrifuge cell culture media at 3000 x *g* for 10 minutes to remove debris. Collect supernatants and assay. Store samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **MIX Diluent Concentrate (10x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute MIX Diluent Concentrate 1:10 with reagent grade water. Store for up to 30 days at 2-8°C.
- **Standard Curve:** Reconstitute the 960 ng of Mouse Myoglobin Standard with 3 ml of MIX Diluent to generate a 320 ng/ml standard solution. Allow the standard to sit for 10 minutes with gentle agitation prior to

making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (320 ng/ml) 1:4 with MIX Diluent to produce 80, 20, 5, 1.25, and 0.313 ng/ml solutions. MIX Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within 30 days.

Standard Point	Dilution	[Mouse Myoglobin] (ng/ml)
P1	Standard (320 ng/ml)	320.0
P2	1 part P1 + 3 parts MIX Diluent	80.00
P3	1 part P2 + 3 parts MIX Diluent	20.00
P4	1 part P3 + 3 parts MIX Diluent	5.000
P5	1 part P4 + 3 parts MIX Diluent	1.250
P6	1 part P5 + 3 parts MIX Diluent	0.313
P7	MIX Diluent	0.000

- **Biotinylated Mouse Myoglobin Antibody (50x):** Spin down the antibody briefly and dilute the desired amount of the antibody 1:50 with MIX Diluent. Any remaining solution should be frozen at -20°C.
- **Wash Buffer Concentrate (20x):** If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. Dilute Wash Buffer Concentrate 1:20 with reagent grade water.
- **SP Conjugate (100x):** Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with MIX Diluent. Any remaining solution should be frozen at -20°C.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20-25°C).
- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
- Add 50 µl of Mouse Myoglobin Standard or sample per well. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
- Wash five times with 200 µl of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µl of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.

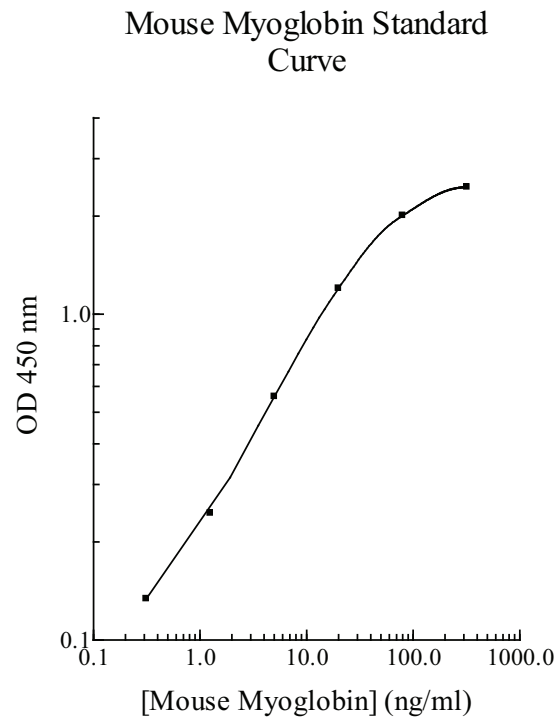
- Add 50 μl of Biotinylated Mouse Myoglobin Antibody to each well and incubate for 1 hour.
- Wash the microplate as described above.
- Add 50 μl of Streptavidin-Peroxidase Conjugate per well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
- Wash the microplate as described above.
- Add 50 μl of Chromogen Substrate per well and incubate for about 20 minutes or till the optimal blue color density develops. Gently tap the plate to ensure thorough mixing and break the bubbles in the well with pipette tip.
- Add 50 μl of Stop Solution to each well. The color will change from blue to yellow.
  Read the absorbance on a microplate reader at a wavelength of 450 nm **immediately**. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Data Analysis

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

Standard Curve

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



Performance Characteristics

- The minimum detectable level of mouse myoglobin is typically ~ 0.3 ng/ml.
- Intra-assay and inter-assay coefficients of variation were 4.1% and 7.1% respectively.

Linearity

	Average Percentage of Expected Value	
Sample Dilution	**Plasma**	**Serum**
No Dilution	99%	100%
1:2	102%	104%
1:4	105%	105%

Recovery

Standard Added Value	1.0 – 80 ng/ml
Recovery %	85 – 109%
Average Recovery %	96.5%

Cross-Reactivity

Species	% Cross Reactivity
Canine	None
Bovine	None
Monkey	10%
Mouse	100%
Rabbit	None
Rat	None
Swine	50%
Human	50%

References

(1) Weller P *et al*. (1984) *EMBO J*. 3: 439-446
(2) Akaboshi E (1985) *Gene* 33: 241-249
(3) Wittenberg JB and Wittenberg BA (2003) *J Exp Biol.* 206(Pt 12):2011-2020
(4) Rahaman MM and Straub AC (2013) *Redox Biology* 1:405-410
(5) Premru V *et al*. (2013) *Ther Apher Dial*. 17(4):391-395
(6) Kristiansen G *et al*. (2011) *J. Biol. Chem.* 286:43417-43428

Version 1.1

Related Products

- ERM8001-1 AssayMax Rat Myoglobin ELISA Kit (Plasma, Serum, Urine, Tissue Extracts, and Cell Culture samples)