ab285235 – Mouse Troponin I ELISA Kit

For the measurement of mouse Troponin I in serum, plasma and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285235

Storage and Stability

The entire unopened kit may be stored at 4°C for 1 month from the date of shipment. If the kit is not used within 1 month, store the items separately according to the following conditions once the kit is received.

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 x 12 strips	-20°C
Standard	2 vials	-20°C
Sample and Standard Diluent	20 mL	+4°C
100X Biotinylated Detection Antibody	120 µL	-20°C
Biotinylated Detection Antibody Diluent	14 mL	+4°C
100X HRP-Streptavidin Conjugate	120 µL	-20°C (In dark)
HRP Conjugate Diluent	14 mL	+4°C
Substrate Reagent	10 mL	+4°C (In dark)
Stop Solution	10 mL	+4°C
25X Wash Buffer	30 mL	+4°C
Plate Sealers	4	RT

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Distilled or deionized water

Reagent Preparation

Prepare reagents within 30 minutes before the experiment. Before using the kit, spin tubes and bring down all components to the bottom of the tubes.

Biotin- detection antibody working solution: Calculate the total volume of the working solution: 0.1 mL / well × quantity of wells with additional 0.1 - 0.2 mL of the total volume. Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.

HRP-Streptavidin Conjugate: Calculate the total volume of the working solution: 0.1 mL / well × quantity of wells with additional 0.1 - 0.2 mL of the total volume. Dilute the HRP-Streptavidin Conjugate with HRP-Streptavidin Conjugate dilution buffer at 1:100 and mix thoroughly.

Wash Buffer: Dilute 30 mL of Concentrated Wash Buffer into 720 mL of Wash Buffer with deionized or distilled water to prepare 750 mL of Wash Buffer.

Δ Note: *If crystals have formed in the concentrate, warm it with 40°C water bath and mix it gently until the crystals have completely dissolved. The solution should be cooled to room temperature before use.*

Standard Preparation

- Centrifuge the standard at 10,000×g for 1 minute.
- Reconstitute the standard by adding 1 mL of Standard/Sample Dilution Buffer to make the 2000 pg/mL standard stock solution. Use within 2 hours after reconstituting.
- Allow solution to sit at room temperature for 10 minutes, then gently vortex to mix completely.
- Prepare 1 mL of 1000 pg/mL top standard by adding 0.5 mL of the above stock solution in 0.5 mL of Standard/Sample Dilution Buffer.
- Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay.
- Suggested standard points are: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL

Sample Preparation

Samples to be assayed within 7 days when stored at 4°C, otherwise samples must be aliquoted and stored at -20°C (≤1 month) or -80°C (≤3 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

Serum: Coagulate the serum for 2 hours at room temperature or overnight at 4°C. Centrifuge at approximately 1000×g for 20 minutes at 2-8°C. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and nonendotoxin.

Plasma: Collect plasma with heparin or EDTA as the anticoagulant. Centrifuge for 15 minutes at 2-8°C at 1000 x g within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Cell lysates: For adherent cells, gently wash the cells with moderate amount of pre-cooled PBS and dissociate the cells using trypsin. Collect the cell suspension into a centrifuge tube and centrifuge for 5 minutes at 1000×g. Discard the medium and wash the cells 3 times with pre-cooled PBS. For each 1×10^6 cells, add 150-250 µL of pre-cooled PBS to keep the cells suspended. Repeat the freeze thaw process several times until the cells are fully lysed. Centrifuge for 10 minutes at 1500xg at 4°C. Remove the cell fragments, collect the supernatant for assay. Avoid repeated freeze-thaw cycles.

Tissue homogenates: It is recommended to get detailed references from the literature before analyzing different tissue types. For general information, hemolysed blood may affect the results, so the tissues should be minced into small pieces and rinsed in ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then homogenized in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant.

Cell culture supernatant: Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°C. Collect the clear supernatant and carry out the assay immediately.

Other biological fluids: Centrifuge samples for 20 minutes at 1000×g at 2 - 8°C. Collect the supernatant and carry out the assay immediately.

Assay Protocol

Δ Note: *Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.*

1. Prepare all reagents, samples and standards as instructed above.

2. Add 100 µL of each standard or samples into appropriate wells. Cover the plate with the plate sealer provided in the kit and incubate for 90 minute at 37°C.

Δ Note: *Solutions should be added to the bottom of the micro-ELISA plate well, avoid touching the inside wall and bubble formation as much as possible.*

3. Remove the liquid out of each well. Do not wash. Immediately add 100 µL of Biotinylated Detection Antibody working solution to each well. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 1 hour at 37°C.
4. Aspirate the solution from each well add 350 µl of 1x wash buffer to each well. Soak for 1~2 minute and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

Δ Note: *A microplate washer can be used in this step and other wash steps.*

5. Add 100 µL of HRP Conjugate working solution to each well. Cover with the Plate sealer. Incubate for 30 minutes at 37°C.
6. Aspirate the solution from each well, repeat the wash process for five times as conducted in step 4.
7. Add 90 µL of Substrate Reagent to each well. Cover with a new plate sealer. Incubate for about 15 minutes at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual colour change, but not more than 30 min.
8. Add 50 µL of Stop Solution to each well.

Δ Note: *Adding the stop solution should be done in the same order as the substrate solution.*

9. Read the absorbance in micro plate reader set to 450 nm.

Calculation:

1. Determine the average of the duplicate readings for each standard and samples then subtract the average zero standard optical density.
2. Plot a four-parameter logistic with standard concentration on the x-axis and OD values on the y-axis.
3. If the samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.
4. If the OD of the sample is under the lowest limit of the standard curve, retest the samples with appropriate dilution.
5. The actual concentration is the concentration obtained by calculated multiplied by the dilution factor.

Technical Support

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