

Mouse PM20D1 ELISA Kit

(Catalog Number: 32700)

For the quantitative determination of mouse PM20D1
concentrations in serum or plasma samples

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INTRODUCTION

PM20D1 is a bidirectional N-fatty-acyl amino acid synthase/hydrolase that regulates the production of N-fatty-acyl amino acids. These metabolites are endogenous chemical uncouplers of mitochondrial respiration. In a UCP1-independent manner, maybe through interaction with mitochondrial transporters, they promote proton leakage into the mitochondrial matrix. PM20D1 may indirectly regulate the bodily dissipation of chemical energy as heat through thermogenic respiration.

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is precoated with affinity purified polyclonal antibody against mouse PM20D1. Standards and samples are pipetted into the wells and any mouse PM20D1 present is bounded by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody against mouse PM20D1 is added to the wells. After washing step to remove any unbound reagents, Streptavidin-horseradish peroxidase (STP-HRP) conjugate is added. After the last wash step, a HRP substrate solution containing 3,3',5,5'-Tetramethylbenzidine (TMB) is added, and color develops in proportion to the amount of mouse PM20D1 bound initially. Color reaction is stopped by 2M H₂SO₄ and the optical density of the wells are determined using a microtiter plate reader at 450nm. Since the increases in absorbance are directly proportional to the amount of captured mouse PM20D1, the unknown sample concentration can be calculated from the standard curve included in each assay.

INTENDED USE

This Mouse PM20D1 ELISA kit is designed for the detection and quantitative measurement of mouse PM20D1 in serum or plasma samples.

REAGENTS SUPPLIED

Each kit is sufficient for one 96-well plate and contains the following components:

1. Microtiter Strips (96 wells), coated with affinity purified polyclonal antibody against mouse PM20D1, sealed
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 30 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against mouse PM20D1, 0.12 mL
5. Mouse PM20D1 standard, 20 ng of recombinant mouse PM20D1, lyophilized
6. 200×STP-HRP solution, 0.06 mL
7. Substrate solution, 12 mL, ready for use
8. Stop solution, 12 mL, ready for use

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips

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2. 96-well plate or manual strip washer
3. Buffer and reagent reservoirs
4. Paper towels or absorbent paper
5. Plate reader capable of reading absorbance at 450 nm
6. Distilled water or deionized water

STORAGE

The kit should be stored at 2-8°C upon receipt, and all reagents should be equilibrated to room temperature before use. Remove any unused antibody-coated strips from the mouse PM20D1 microtiter plate, return them to the foil pouch and re-seal. Once opened, the strips may be stored at 2-8°C for up to one month.

PREPARATION OF REAGENTS

Bring all reagents and materials to room temperature before assay.

A. 1×Assay buffer

Prepare 1×Assay buffer by mixing the 5×Assay buffer (30 mL) with 120 mL of distilled water or deionized water. If precipitates are observed in the 5×Assay buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Assay buffer may be stored at 2-8°C for up to one month.

B. 1×Wash buffer

Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

C. 1×Detection antibody solution

Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required for each well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to 2-8°C immediately after the necessary volume is removed.

D. 1×STP-HRP solution

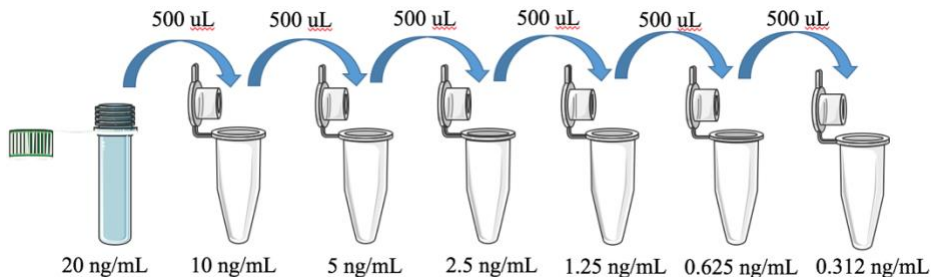
Spin down the 200×STP-HRP solution briefly and dilute the desired amount of the 200×STP-HRP solution 1:200 with 1×Assay buffer, 100 µL of the 1×STP-HRP solution is required for each well. Prepare only as much 1×STP-HRP solution as needed. Return the 200×STP-HRP solution to 2-8°C immediately after the necessary volume is removed.

PREPARATION OF STANDARDS AND SAMPLES

Mouse PM20D1 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 20 ng/mL. Allow standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of



1×Assay buffer to 10, 5, 2.5, 1.25, 0.625, 0.312 ng/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and stored at -20°C for one month. Avoid repeating freezing/thawing cycles.

Sample Preparation:

Serum or plasma sample generally requires a **50-fold** dilution in the 1×Assay buffer. It is recommended that the users establish their own dilution factors based on the concentration range of their samples.

ASSAY PROCEDURE

It is recommended that all standards and samples be assayed in duplicate.

1. Add 100 µL of standard or sample to its corresponding well, incubate at room temperature for 1 hour.
2. Discard the content and tap the plate on a clean paper towel to remove residual solution in each well. Add 300 µL of 1×Wash buffer to each well and incubate for 1 minute. Discard the 1×Wash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total of 3 times.
3. Add 100 µL of 1×Detection antibody solution to each well, incubate at room temperature for 1 hour.
4. Wash each well 3 times as described in step 2.
5. Add 100 µL of 1×STP-HRP solution to each well, incubate at room temperature for 20 minutes.
6. Wash each well 4 times as described in step 2.
7. Add 100 µL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
8. Add 100 µL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
9. Measure absorbance of each well at 450 nm immediately.

CALCULATION

1. Subtract the absorbance of the blank from that of standards and samples.

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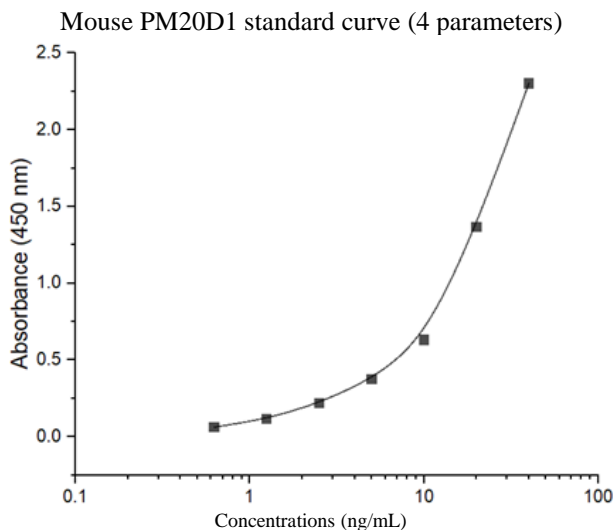
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2. Generate a standard curve by plotting the absorbance obtained (y-axis) against mouse PM20D1 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine mouse PM20D1 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

The following standard curve is provided for demonstration only. The standard curve should be generated for each set of sample assay.

Mouse PM20D1 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.096	0
0.312	0.128	0.032
0.625	0.161	0.065
1.25	0.23	0.134
2.5	0.371	0.275
5	0.609	0.513
10	1.116	1.07
20	2.191	2.095



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ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of mouse PM20D1 that can be measured by this assay is 0.156 ng/mL.

B. Specificity:

No cross reactivity or interference was observed when test with recombinant human PM20D1 protein.

C. Precision:

Intra-assay Precision (Precision within one assay)

One sample of known concentration were tested 8 times on same plate.

C.V.: 3.8%

Inter-assay Precision (Precision between assays)

One sample of known concentration were tested in 8 separate assays.

C.V.: 4.9%

D. Spike and Recovery

Serum samples are measured by adding 90 μ L of sample and 10 μ L of spike stock solution calculated to yield the intended 0, 1, 2 or 4 ng/mL spike concentration.

Spike level	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
Low spike (1 ng/mL)	0.94	1.04	110.7
Medium spike (2 ng/mL)	1.91	2.21	115.8
High spike (4 ng/mL)	4.1	3.83	93.4

E. Linearity and Recovery

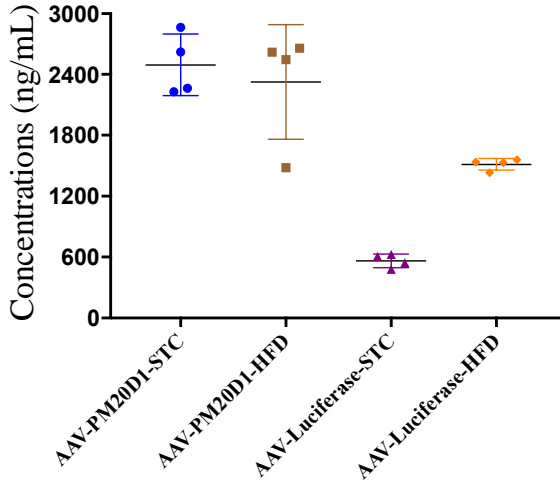
To assess the linearity of the assay, samples spiked with high concentrations of mouse PM20D1 were serially diluted with the 1 \times Assay buffer to produce samples with values within the dynamic range of the assay.

Sample 1

Dilution	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1:2	13.69	14.37	95.3
1:4	8.39	7.76	92.5
1:8	3.86	3.29	117.2

F. Validation

C57BL/6 mice were fed with high-fat diet or standard chow along with AAV-PM20D1 or AAV-luciferase injection for 18 weeks, mouse serum was collected for the measurement of PM20D1 levels.



SUMMARY OF ASSAY PROCEDURE

