Mouse PM20D1 ELISA Assay Kit

Catalog Number: PMD11-K01

For the quantitative determination of mouse PM20D1 concentration in serum and plasma samples.

This package insert must be read in its entirety before using this product

1 x 96 Wells

For Research Use Only (RUO). Not for use in clinical, diagnostic or therapeutic procedures.

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ASSAY BACKGROUND

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 an 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.

ASSAY PRINCIPLE

This assay is a quantitative sandwich ELISA. The microplate is pre-coated with a polyclonal antibody specific for mouse PM20D1. Standards and samples are pipetted into the wells and any human PM20D1 present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase biotin-labelled polyclonal antibody specific for mouse PM20D1 is added to the wells. After wash step to remove any unbound reagents, streptavidin-HRP conjugate (STP-HRP) is added. After the last wash step, an HRP-substrate solution is added and colour develops in proportion to the amount of mouse PM20D1 bound initially. The assay is stopped and the optical density of the wells determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured mouse PM20D1, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

The Eagle Biosciences Mouse PM20D1 ELISA Assay Kit is designed for quantification of mouse PM20D1 in serum and plasma samples. The Mouse PM20D1 ELISA Assay Kit is for research use only and not to be used for diagnostic purposes.

REAGENTS SUPPLIED

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

1. Micro-titre Strips (96 wells)-Coated with a 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 in a buffered protein base, lyophilised
6. 200×STP-HRP solution- 0.06 ml
7. Substrate solution- 12 ml, ready for use.
8. Stop solution- 12 ml, ready for use.
9. Plate cover-1.

OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

1. Pipettes and pipette tips.
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 absorbency 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 microplate, 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 l of the 1×Detection antibody solution is required per 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 l of the 1×STP-HRP solution is required per 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 STANDRADS AND SAMPLES

Mouse PM20D1 standards: Reconstitute the lyophilised standard with 1 ml of 1×Assay buffer to generate a standard stock solution of 20 ng/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.

Prepare serially diluted standards using 1×Assay buffer as follows:

Standard volume	Volume of 1×Assay buffer	Concentration
20 ng/ml stock	-	20 ng/ml
500 l of 20 ng/ml	500 l	10 ng/ml
500 l of 10 ng/ml	500 l	5 ng/ml
500 l of 5 ng/ml	500 l	2.5 ng/ml
500 l of 2.5 ng/ml	500 l	1.25 ng/ml
500 l of 1.25 ng/ml	500 l	0.625 ng/ml
500 l of 0.625 ng/ml	500 l	0.312 ng/ml

1×Assay buffer serves as the zero standard (0 ng/ml).

The reconstituted standard stock should be aliquoted and stored at -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample preparation

Serum or plasma sample is generally required at least 100-fold dilution in the 1×Assay buffer.

Assay procedure

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

1. Add 100 µl of standard or sample to its corresponding well, seal the plate with a plate cover. 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 1x Wash buffer to each well and incubate for 1 minute. Discard the 1xWash buffer and tap the plate on a clean paper towel to remove residual wash buffer. Repeat the wash step for a total 3 washes.
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 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.
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. 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. A standard curve should be generated for each set of sample assay.

Mouse PM20D1(ng/ml)	Absorbance (450 nm)	Blanked Absorbance
0	0.075	0
0.312	0.107	0.032
0.625	0.141	0.066
1.25	0.205	0.13
2.5	0.35	0.275
5	0.596	0.521
10	1.189	1.114
20	2.054	1.979

Mouse PM20D1 standard curve （4 parameterS)

ASSAY CHARACTERISTICS

A. Sensitivity:

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

B. Specificity:

Cross reactivity with recombinant human PM20D1 protein.

C. Precision:

Intra-assay Precision (Precision within an assay)
Two samples of known concentration were tested 8 times on one plate.
CV%: 3.8%
Inter-assay Precision (Precision between assays)
Two samples of known concentration were tested in 8 separate assays.
CV%: 4.9%

D. Spiking:
Serum samples were assayed by adding 90 µl of sample and 10 µl 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)	4.10	3.83	93.4

E. Linearity:
To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse PM20D1 were serially diluted with the 1×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
Mouse serum from 18 weeks after AAV-PM20D1 overexpressed fed with High-fat diet or Standard chow and control group (AAV-luciferase).

Summary of Assay Procedure

Add 100 µl of standard or sample to each well.

Incubate at room temperature for 1 hour.

Aspirate and wash each well three times.

Add 100 µl of 1xDetection antibody solution to each well.

Incubate at room temperature for 1 hour.

Aspirate and wash each well three times.

Add 100 µl of 1x STP-HRP solution to each well.

Aspirate and wash each well four times.

Add 100 µl of Substrate solution to each well.

Incubate at room temperature for 15 minutes.

Add 100 µl of Stop solution to each well.

Measure absorbance of each well at 450 nm.

Calculation

Warranty Information

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For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.

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