ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Human PM20D1 ELISA Kit

(Catalog Number: 31700)

For the quantitative determination of human 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 human PM20D1. Standards and samples are pipetted into the wells and any human PM20D1 present is bounded by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody against human 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 human PM20D1 bound initially. Color reaction is stopped by 2M H_2SO_4 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 human PM20D1, the unknown sample concentration can be calculated from the standard curve included in each assay.

INTENDED USE

This Human PM20D1 ELISA kit is designed for the detection and quantitative measurement of human 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 human 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 human PM20D1, 0.12 mL
5. Human PM20D1 standard, 2000 pg of recombinant human 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

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 human 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.

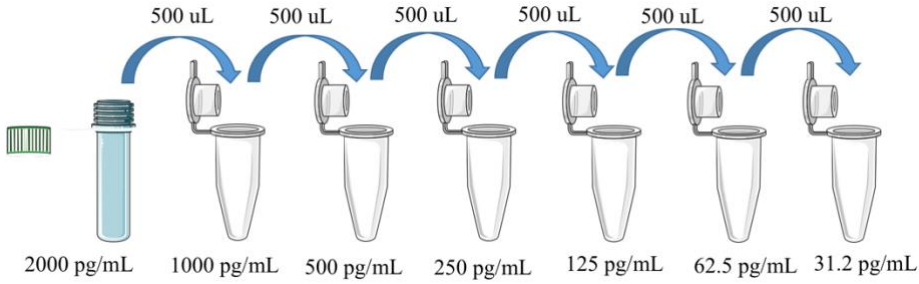
D. 1×STP-HRP solution

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

Human PM20D1 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 2000 pg/mL. Allow standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of

1×Assay buffer to 1000, 500, 250, 125, 62.5, 31.2 pg/mL tubes. Use the standard stock solution to produce a serial dilution as shown below.



1×Assay buffer serves as the zero standard (0 pg/mL). The reconstituted standard stock should be aliquoted and stored at -20ºC 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 2 hours.
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 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.

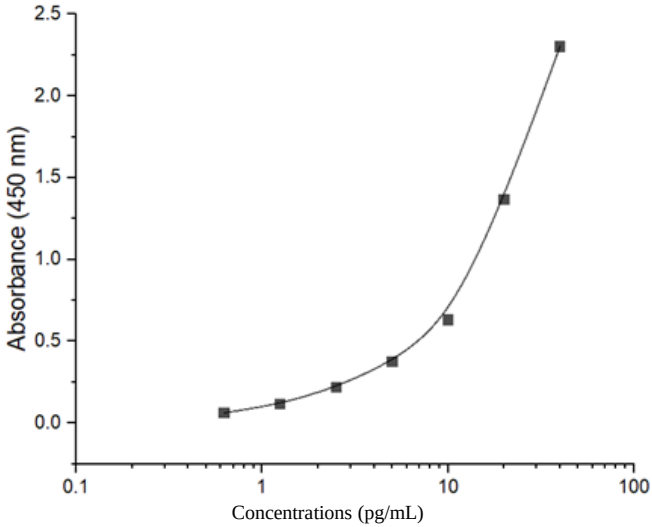
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against human PM20D1 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine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

Human PM20D1(pg/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.103	0
31.2	0.126	0.023
62.5	0.161	0.058
125	0.237	0.134
250	0.421	0.318
500	0.747	0.644
1000	1.134	1.031
2000	2.12	2.017

Human PM20D1 standard curve (4 parameters)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of human PM20D1 that can be measured by this assay is 7.8 pg/mL.

B. Specificity

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

C. Precision

Intra-assay Precision (Precision within an assay)
One sample of known concentration were tested 8 times on one plate.

Sample	Mean (pg/mL)	SD	CV (%)
1	32723.26	1145.522	3.5

Inter-assay Precision (Precision between assays)
One sample of known concentration were tested in 8 separate assays.

Sample	Mean (pg/mL)	SD	CV (%)
2	4568.75	160.22	3.5

D. Spike and Recovery

Serum samples are measured by adding 90 µL of sample and 10 µL of spike stock solution calculated to yield the intended 0, 50, 400 or 800 pg/mL spike concentration.

Spike level	Expected (pg/mL)	Observed (pg/mL)	Recovery (%)
Low spike (50 pg/mL)	49.83	47.83	95
Medium spike (400 pg/mL)	396.5	357.33	90.1
High spike (800 pg/mL)	679.83	724	106.5

E. Linearity and Recovery

To assess the linearity of the assay, samples containing high concentrations of human 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 (pg/mL)	Expected (pg/mL)	Recovery (%)
1:100	3072.46	27850.9	110.3
1:200	30770	27850.9	110.5

SUMMARY OF ASSAY PROCEDURE

Add 100 µL of standard or sample to each well.
↓
Incubate at room temperature for 2 hours.
↓
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 1xSTP-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