

Mouse Major Urinary Protein-1 ELISA Kit

(Catalog Number: 32150)

For the quantitative determination of mouse major urinary
protein-1 concentrations in serum or plasma

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INTRODUCTION

Major urinary protein 1 (MUP-1), also known as Mup7, Up-1, Ltn-1, Mup-1, Mup-a, Mup10 and Lvtn-1, is a low molecular weight secreted protein produced predominantly from the liver¹. Structurally, it belongs to the lipocalin family, which carries small hydrophobic ligands such as pheromones. It has been demonstrated that MUP-1 is an important player in regulating energy expenditure and metabolism in mice. In both dietary and genetic obese mice, the circulating concentrations of MUP-1 were markedly decreased compared with the lean controls. Replenishment of recombinant MUP-1 led to improved glucose tolerance and insulin sensitivity, as well as increased energy expenditure and locomotor activity in db/db diabetic mice². It was suggested that MUP-1 also regulates systemic glucose and/or lipid metabolism through the paracrine/autocrine regulation of the hepatic gluconeogenic and/or lipogenic programs, respectively³.

PRINCIPLE OF THE ASSAY

This assay is a sandwich enzyme-linked immunosorbent assay (ELISA) designed for the quantitative detection of mouse MUP-1 in samples. The microtiter plate is pre-coated with antibody specific to mouse MUP-1. Standards and samples are pipetted into the wells and any mouse MUP-1 present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for mouse MUP-1 is added to the wells. After a final wash step, an HRP substrate solution is added and color develops in proportion to the amount of mouse MUP-1 bound initially. The assay is stopped, and the optical density of the wells is determined using a microplate reader. Since the increases in absorbance are directly proportional to the amount of captured mouse MUP-1, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Mouse MUP-1 ELISA kit is designed for quantification of mouse MUP-1 in serum and 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 a polyclonal antibody against mouse MUP-1, sealed
2. 10×Wash buffer, 40 mL
3. 5×Assay buffer, 40 mL
4. 100×Detection antibody solution, a polyclonal antibody against mouse MUP-1 conjugated with horseradish peroxidase, 0.12 mL
5. Mouse MUP-1 standard, 40 ng of recombinant mouse MUP-1, lyophilized
6. Substrate solution, 12 mL, ready for use
7. Stop solution, 12 mL, ready for use

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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 MUP-1 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 (40 mL) with 160 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

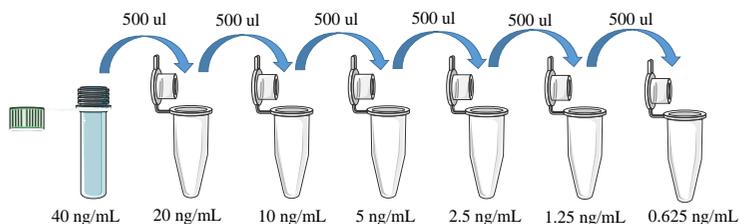
Prepare 1×Wash buffer by mixing the 10×Wash buffer (40 mL) with 360 mL of distilled water or deionized water. If precipitates are observed in the 10×Wash buffer bottle, warm the bottle in a 37°C water bath until the precipitates disappear. The 1×Wash buffer may be stored at 2-8°C for up to one month.

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 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.

PREPARATION OF STANDARDS AND SAMPLES

Mouse MUP-1 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 40 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 shown below.



1×Assay buffer serves as the zero standard (0 ng/mL). The reconstituted standard stock should be aliquoted and frozen at -20°C for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires a **4000-fold** dilution in this assay. A two-step dilution is suggested. Step1-Add 10 μL of sample into 990 μL of 1×Assay buffer to give a 100-fold diluted sample solution. Step 2-Add 10 μL of the 100-fold diluted sample into 390 μL of 1×Assay buffer to give a 4000-fold diluted sample. Dilution factors for cellular extracts and culture media need to be optimized by the user. 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 per 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 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 4 times as in step 2.
5. Add 100 μL of Substrate solution to each well, incubate at room temperature for 15 minutes. **Protect from light.**
6. Add 100 μL of Stop solution to each well, gently tap the plate frame for a few seconds to ensure thorough mixing.
7. 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 MUP-1 concentrations (x-axis). The best fit line can be generated with any curve-

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fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.

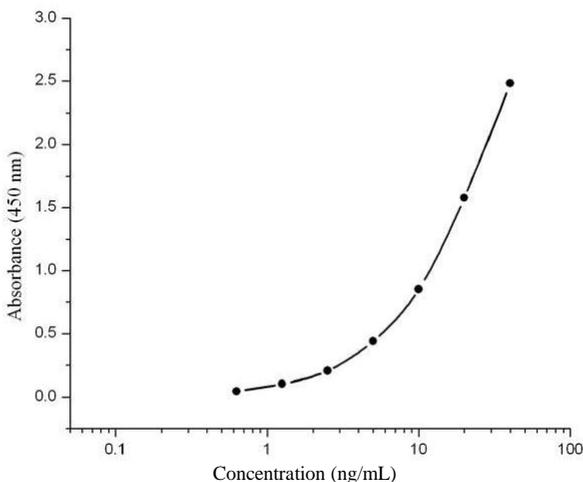
- Determine MUP-1 concentration of samples from standard curve and multiply the value by the dilution factor.

TYPICAL STANDARD CURVE

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

MUP-1 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.071	0
0.625	0.117	0.046
1.25	0.174	0.103
2.5	0.279	0.208
5	0.512	0.441
10	0.927	0.852
20	1.65	1.579
40	2.555	2.484

Mouse MUP-1 standard curve (4-parameter)



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ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of mouse MUP-1 that can be detected by this assay is 0.625 ng/mL.

B. Specificity

The antibodies used in this assay are specific to mouse MUP-1.

REFERENCES

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3. Zhou Y, Jiang L, Rui L. (2009) *J Biol Chem.* 284(17):11152-9.

SUMMARY OF ASSAY PROCEDURE

