Mouse Major urinary protein-1 Immunoassay Kit

Catalogue Number: 31150

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

This package insert must be read in its entirety before using this product
Use only the current version of Product Data Sheet enclosed with the kit

Version: 3.0

FOR RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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INTRODUCTION

Major urinary protein 1 (Mup1), 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 liver. [1] Structurally it belongs to the lipocalin family, which carries small hydrophobic ligands such as pheromones. It has been demonstrated that Mup1 is an important player in regulating energy expenditure and metabolism in mice. In both dietary and genetic obese mice, the circulating concentrations of Mup1 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 mice. [2] It was suggested that Mup1 also regulates systemic glucose and/or lipid metabolism through the paracrine/autocrine regulation of the hepatic gluconeogenic and/or lipogenic programs, respectively. [3]

PRINCIPLE OF THE ASSAY

This assay is a sandwich ELISA designed for the quantitative detection of mouse Mup1 in samples. The immunoplate is pre-coated with antibody specific to mouse Mup1. Standards and samples are pipetted into the wells and any mouse Mup1 present is bound by the immobilized antibody. After washing away any unbound substances, a horseradish peroxidase (HRP)-linked polyclonal antibody specific for mouse Mup1 is added to the wells. After a final wash step, an HRP substrate solution is added and colour develops in proportion to the amount of mouse Mup1 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 Mup1, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Mup1 ELISA kit is designed for quantification of mouse Mup1 in serum and plasma samples.

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 Mup1, sealed.
2. (10×) Wash buffer -40 ml.
3. (5×) Assay buffer -40 ml.
4. (100×) Detection antibody solution -A polyclonal antibody against mouse Mup1 conjugated with horseradish peroxidise, 0.12 ml.
5. Mouse Mup1 standard-40 ng of recombinant mouse Mup1 in a buffered protein base, lyophilised.
6. Substrate solution- 12 ml, ready for use.
7. 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 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 Mup1 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 (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 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.

PREPARATION OF STANDRADS AND SAMPLES

Mup1 Standards: Reconstitute the lyophilised 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 follows:

Standard volume	Volume of 1x Assay buffer	Concentration
40 ng/ml stock	-	40 ng/ml
250 l of 40 ng/ml stock	250 l	20 ng/ml
250 l of 20 ng/ml std.	250 l	10 ng/ml
250 l of 10 ng/ml std.	250 l	5 ng/ml
250 l of 5 ng/ml std.	250 l	2.5 ng/ml
250 l of 2.5ng/ml std.	250 l	1.25 ng/ml
250 l of 1.25 ng/ml std.	250 l	0.625 ng/ml

1x Assay buffer serves as the zero standard (0 ng/ml). The reconstituted standard stock should be aliquoted and frozen 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 a 4000-fold dilution in this assay. A two-step dilution is suggested. Step1: Add 10 l of sample into 990 l of 1× Assay buffer to give a 100-fold diluted sample solution. Step 2- Add 10 l of the 100-fold diluted sample into 390 l 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.

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

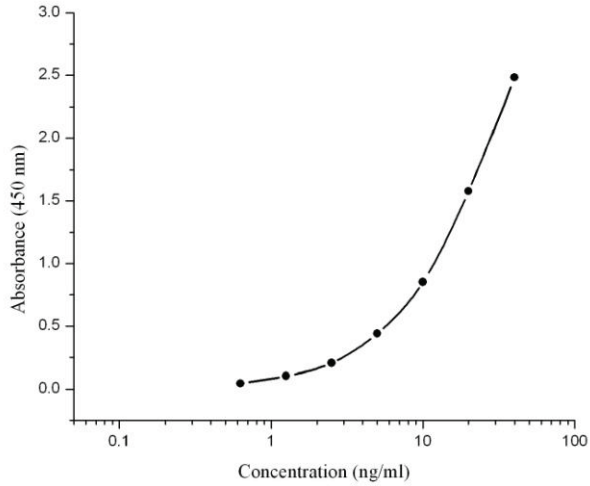
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against Mup1 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine Mup1 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.

Mup1 (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)



ASSAY CHARACTERISTICS

A. Sensitivity: The lowest level of mouse Mup1 that can be detected by this assay is 0.625 ng/ml.

B. Specificity: The antibodies used in this assay are specific to mouse Mup1.

REFERENCES:

1. McIntosh, I., and Bishop, J. O. (1989) *Mol. Cell. Biol.* 9, 2202–2207.
2. Hui X, Zhu W, Wang Y, et al. (2009) *J Biol Chem*. 284(21):14050-7.
3. Zhou Y, Jiang L, Rui L. (2009) *J Biol Chem*. 284(17):11152-9.

SUMMARY OF ASSAY PROCEDURE

Add 100 µl of Standard or sample per well.

⊠

Incubate at room temperature for 1 hour.

⊠

Aspirate and wash each well three times.

⊠

Add 100 µl of 1× Detection antibody solution to each well.

⊠

Incubate at room temperature for 1 hour.

⊠

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