ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Mouse CYSTM1 ELISA Kit

(Catalog Number: 32280)

For the quantitative determination of mouse CYSTM1 concentrations in serum or plasma

IMD (Hong Kong)
Address: Unit 513, 5/F, Biotech Centre 2, No. 11 Science Park West Avenue, Hong Kong Science Park, Sha Tin, Hong Kong
Website: www.immunodiagnostics.com.hk
Email: info@immunodiagnostics.com.hk
Tel: (+852) 3502 2780

IMD (Canada)
Address: 3330 Bayview Avenue, Block #6, Toronto, M2M 3R8, Ontario, Canada
Email: info@immunodiagnostics.ca
Tel: +1-437-886-5136

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INTRODUCTION

Cysteine-rich transmembrane module containing 1 (CYSTM1) also known as C5orf32, ORF1-FL49, belongs to CYSTM family which is a part of tail-anchored membrane proteins in eukaryotes. It consists of 104 and 97 amino acids in mice and humans respectively. CYSTM1 is a transmembrane protein and based on Gene Ontology (GO) the function of CYSTM1 is classified as neutrophil degranulation. CYSTM1 was also reported as one of the candidate biomarkers for Huntington's disease[1,2].

PRINCIPLE OF THE ASSAY

This assay is a quantitative sandwich enzyme-linked immunosorbent assay (ELISA). The microtiter plate is pre-coated with a polyclonal antibody specific for mouse CYSTM1. Standards and samples are pipetted into the wells and any mouse CYSTM1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody specific for mouse CYSTM1 is added to the wells. After wash step to remove any unbound reagents, streptavidin-horseradish peroxidase conjugate (STP-HRP) is added. After the last wash step, an HRP-substrate solution is added, and color develops in proportion to the amount of mouse CYSTM1 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 CYSTM1, the unknown sample concentration can be interpolated from a reference curve included in each assay.

INTENDED USE

This Mouse CYSTM1 ELISA kit is designed for quantification of mouse CYSTM1 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 CYSTM1, sealed
2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 30 mL
4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against mouse CYSTM1, 0.12 mL
5. Mouse CYSTM1 standard, 10 ng of recombinant mouse CYSTM1, 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 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 CYSTM1 microtiter plate, return them to the foil pouch and reseal. 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 (50 mL) with 450 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

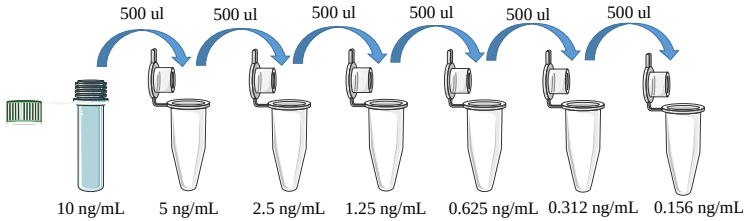
Spin down the 100×Detection antibody solution briefly and dilute the desired amount of the antibody 1:100 with 1×Assay buffer, 100 µL of the 1×Detection antibody solution is required per well. Prepare only as much 1×Detection antibody solution as needed. Return the 100×Detection antibody solution to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

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 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 STANDARDS AND SAMPLES

Mouse CYSTM1 Standards: Reconstitute the lyophilized standard with 1 mL of 1×Assay buffer to generate a standard stock solution of 10 ng/mL. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Pipette 500 µL of 1×Assay buffer to 5, 2.5, 1.25, 0.625, 0.312, 0.156 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 -20ºC for one month. Avoid repeating freezing/thawing cycles. Please do not store the diluted standard solutions.

Sample Preparation:

Serum or plasma sample generally requires at least **3-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, seal the plate with a plate cover. 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 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 CYSTM1 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or loglog curve fitting can be used for calculation.

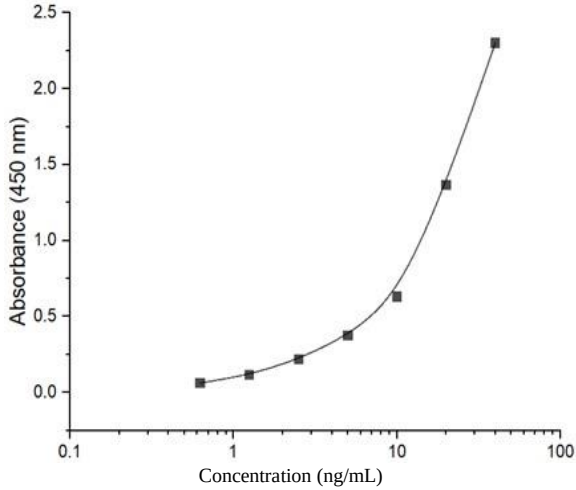
3. Determine mouse CYSTM1 concentration of samples from standard curve and multiply the value by the dilution factor.

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 CYSTM1(ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.101	0
0.156	0.15	0.049
0.312	0.207	0.106
0.625	0.308	0.207
1.25	0.582	0.481
2.5	0.936	0.85
5	1.595	1.494
10	2.544	2.443

Mouse CYSTM1 standard curve（4 parameters）



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of mouse CYSTM1 that can be measured by this assay is 0.156 ng/mL.

B. Specificity

No cross reaction with human CYSTM1.

C. Precision

Intra-assay Precision (Precision within an assay)
Two samples of known concentration were tested 8 times on one plate. C.V.: 5%

Inter-assay Precision (Precision between assays)
Two samples of known concentration were tested in 8 separate assays. C.V.: 5.5%

D. Spiking

Spike level	Expected (ng/mL)	Observed (ng/mL)	Recovery (%)
Low spike	0.5	0.41	82.6
High spike	1	0.842	84.2

E. Linearity

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse CYSTM1 were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

Dilution	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1/2	0.68	0.675	100.7
1/4	0.31	0.337	92
1/8	0.14	0.168	83.3

REFERENCES

1. Mastrokolias, A, et al. (2015) Eur. J. of Hum Genet. 23(10): 1349-1356.
2. Xu, Y. et al. (2018) Plant Cell Physiol. 59(2): 423-438.

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