ImmunoDiagnostics Limited

FOR RESEARCH USE ONLY

Mouse STC2 ELISA Kit

(Catalog Number: 32C040)

For the quantitative determination of mouse STC2 concentrations in serum, plasma and cell culture supernate samples

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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TABLE OF CONTENTS

INTRODUCTION

Stanniocalcin 2 (STC2) is a glycosylated, disulfide-linked, homodimeric hormone initially identified in the corpus of stannous--a small endocrine organ of bony fish [1, 2]. In mammals, STC2 is widely expressed in different tissues as well as in tumor cells. STC2 has been implicated in diverse biological processes, including calcium and phosphate regulation, cytoprotection, cell development, and angiogenesis [3], and is a potential prognostic marker for variety of cancers [4, 5]. STC2 overexpression in MC3T3 cells facilitated osteoblast differentiation and mineralization through regulation of ERK1/2 phosphorylation, suggesting its involvement in bone metabolism [6]. Additionally, STC2 has been shown to attenuate ovarian progesterone biosynthesis via PKA pathway, accompanied with the inhibition of follicle-stimulating hormone (FSH)-induced Cyp1a1 and 3β-hydroxysteroid dehydrogenase expression. Furthermore, STC2 is a potential metabolic hormone involved in regulation of glucose homeostasis. In both brown adipose tissue and liver of mice, STC2 expression is induced by fasting, and treatment with recombinant STC2 increases glycogen levels in the fasting state, and exerts opposite effects on gluconeogenesis in rat hepatocytes in the fasting and fed states [7, 8].

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 mouse STC2. Standards and samples are pipetted into the wells and any mouse STC2 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-labelled polyclonal antibody against mouse STC2 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, an HRP substrate solution 3,3′,5,5′-Tetramethylbenzidine (TMB) is added, and color develops in proportion to the amount of mouse STC2 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 mouse STC2, the unknown sample concentration can be calculated from the standard curve included in each assay.

INTENDED USE

This mouse STC2 ELISA kit is designed for the detection and quantitative measurement of mouse STC2 in serum, plasma or cell culture supernate 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 mouse STC2, sealed
2. 10×Wash buffer, 50 mL
3. 5×Assay buffer, 20 mL

4. 100×Detection antibody solution, a biotin labelled polyclonal antibody against mouse STC2, 0.12 mL
5. Mouse STC2 standard, 10 ng of recombinant mouse STC2, 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

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 srips from the mouse STC2 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 (20 mL) with 80 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 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.

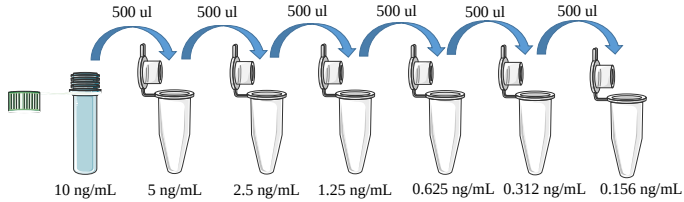
D. 1×STP-HRP solution

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 STANDRADS AND SAMPLES

Mouse STC2 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. Prepare serially diluted standards using 1×Assay buffer as follows:



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 a **16-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 should 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 of 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

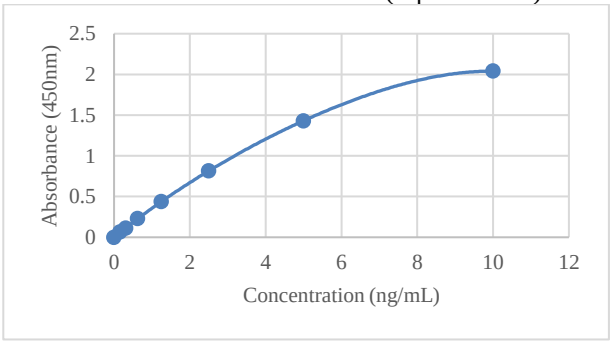
1. Subtract the absorbance of the blank from that of standards and samples.
2. Generate a standard curve by plotting the absorbance obtained (y-axis) against mouse STC2 concentrations (x-axis). The best fit line can be generated with any curve-fitting software by regression analysis. Any curve of 4-parameter or log-log curve fitting can be used for calculation.
3. Determine mouse STC2 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 STC2 (ng/mL)	Absorbance (450 nm)	Blanked Absorbance
0	0.117	0
0.156	0.184	0.067
0.312	0.231	0.114
0.625	0.347	0.23
1.25	0.555	0.438
2.5	0.933	0.816
5	1.548	1.431
10	2.158	2.041

Mouse STC2 standard curve (4-parameters)



ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of mouse STC2 that can be measured by this assay is 0.156 ng/mL.

B. Precision

Intra-assay Precision (Precision within an assay)

Two samples of known concentration were tested 8 times on one plate.

Sample	Mean (ng/mL)	SD (ng/mL)	C.V. (%)
1	17.27	1.40	8.13
2	19.14	1.72	9.01

Inter-assay Precision (Precision between assays)

Two samples of known concentration were tested in 8 separate assays.

Sample	Mean (ng/mL)	SD (ng/mL)	C.V. (%)
1	14.68	1.30	8.84
2	16.45	1.64	9.99

C. Linearity and Recovery

To assess the linearity of the assay, samples spiked with high concentrations of mouse STC2 were serially diluted with the 1×Assay buffer to produce samples with values within the dynamic range of the assay.

Sample1

Dilution	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1:8	19.72	19.72	100.0
1:16	10.69	9.86	108.4
1:32	5.53	4.93	112.2
1:64	2.60	2.46	105.4

Sample 2

Dilution	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
1:8	65.11	65.11	100.0
1:16	37.50	32.56	115.2
1:32	19.53	16.28	120.0
1:64	6.62	8.14	81.4

D. Spike and recovery

Serum samples were assayed by adding 90 µL of sample and 10 µL of spike stock solution calculated to yield the intended 0, 0.625, 2.5 or 10 ng/mL spike concentration.

Spike level	Measured (ng/mL)	Expected (ng/mL)	Recovery (%)
High spike (10 ng/mL)	12.73	11.08	114.9

Medium spike (2.5 ng/mL)	1.97	2.41	81.7
Low spike (0.625 ng/mL)	0.87	0.78	112.3

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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 1x STP-HRP solution to each well.
↓
Incubate at room temperature for 20 minutes
↓
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