Datasheet for ABIN367853

CST3 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CST3
Reactivity:	Mouse
Method Type:	Sandwich ELISA
Detection Range:	2.5-160 ng/mL
Minimum Detection Limit:	2.5 ng/mL
Application:	ELISA

Product Details

Purpose:	This assay employs the quantitative sandwich enzyme immunoassay technique.
Sample Type:	Serum, Plasma, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of Mouse CST3.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	0.819 ng/mL
Components:	• Assay plate (12 × 8 coated Microwells) • Standard (freeze dried)

Product Details

- Biotin-antibody (100 × concentrate)
- HRP-avidin (100 × concentrate)
- Biotin-antibody Diluent
- HRP-avidin Diluent
- Sample Diluent
- Wash Buffer (25 × concentrate)
- TMB Substrate
- Stop Solution
- Adhesive Strip (for 96 wells)
- Instruction manual

Target Details

Target:	CST3
Alternative Name:	Cystatin C (Cys-C) (CST3 Products)
Background:	Synonyms: ARMD11, MGC117328, OTTHUMP00000164181|OTTHUMP00000164182|bA218C14.4 (cystatin C)|cystatin 3|gamma-trace|neuroendocrine basic polypeptide|post-gamma-globulin
HGNC:	2475
UniProt:	P21460

Application Details

Application Notes:

- The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
- Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination.
- Grossly hemolyzed samples are not suitable for use in this assay.
- If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
- Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
- Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
- Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products.

	• Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit. • Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
Comment:	Detection wavelength: 450 nm Information on standard material: Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative. Information on reagents: In most cases the stop solution provided is 1 N H2SO4. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA. Information on antibodies: The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A
Sample Volume:	100 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	Antibody specific for CST3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CST3 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for CST3 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CST3 bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	• **Biotin-antibody (1×)** - Centrifuge the vial before opening.

Application Details

Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10µL of Biotin-antibody + 990µL of Biotin-antibody Diluent.

- **HRP-avidin (1×)** - Centrifuge the vial before opening.
  HRP-avidin requires a 100-fold dilution. The suggested dilution is 10µL of HRP-avidin + 990µL of HRP-avidin Diluent.
- **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×).
- **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s.
  Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 200pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.
  Pipette 250µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (200pg/mL). Sample Diluent serves as the zero standard (0ng/mL).

Note:

- Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit.
- Bring all reagents to room temperature (18-25°C) before use for 30 min.
- Prepare fresh standard for each assay. Use within 4 hours and discard after use.
- Making serial dilution in the wells directly is not permitted.
- Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL when pipetting.
- It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.

Assay Precision:	Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision. Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision. • Intra-assay: CV% less than 8% • Inter-assay: CV% less than 10%
Restrictions:	For Research Use only

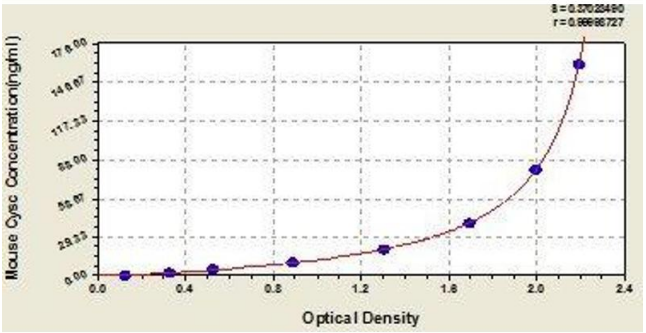
Handling

Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing

Handling

	protection when using this material.
Handling Advice:	• The kit should not be used beyond the expiration date on the kit label. • Do not mix or substitute reagents with those from other lots or sources. • If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay. • Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding. • This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Validation report #029606 for ELISA (ELISA)



ELISA

Image 1. Typical standard curve

Validation report #029704 for ELISA (ELISA)

Successfully validated (ELISA (ELISA))

by Celplor LLC

Report Number: 029704

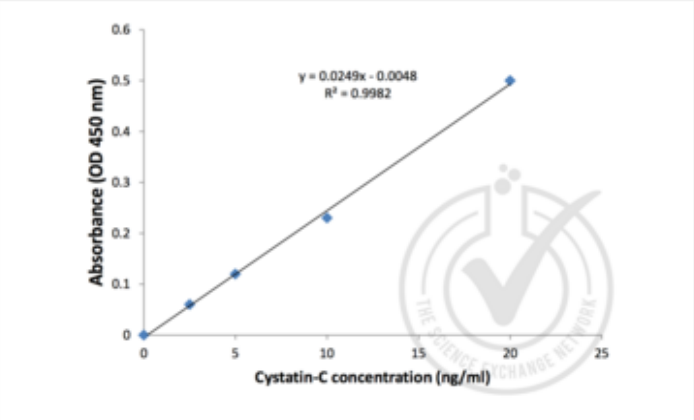
Date: May 16 2014

Lot Number:	R21184078
Method validated:	ELISA (ELISA)
Positive Control:	Mouse serum
Negative Control:	Horse serum (non-reactive species)
Notes:	Signal was detected in positive control and not in negative control.
Primary Antibody:	- Antigen: Cystatin C (CST3) - Catalog number: ABIN367853 - Supplier: Cusabio - Supplier catalog number: csb-e08386m - Lot number: R21184078
Controls:	• Positive control: Normal mouse serum (Jackson ImmunoResearch, Cat# 015-000-001, Lot# 112574) • Negative control: Normal horse serum (In house stock from healthy horse) • Spike control: Standard spiked into sample diluent provided in kit.
Protocol:	• 1. All reagents in the ELISA kit were brought up to room temperature (RT) before use. • 2. 100 µL of each sample was added per well to the micro ELISA plate well. All samples and standards were assayed in triplicate. Plate was covered with sealer (provided in kit) and incubated for 2h at 37°C. • 3. After incubation, liquid in each well was removed by suction. • 4. 100 µL of Biotin-antibody (1X) was added per well. Plate was covered with sealer and incubated for 60 min at 37°C. • 5. Wells were washed with 200 µL of wash buffer three times. Each wash involved fully aspirating the liquid from each well by pipette. After the last wash the plate was inverted against clean absorbent paper to remove any remaining liquid. • 6. 100 µL of HRP-avidin (1x) was added per well. The plate was covered with sealer and incubated for 60 mins at 37°C. • 7. Wells were washed with 200 µL of wash buffer five times same as step 5. • 8. 90 µL of TMB Substrate was added to each well and the plate was covered with a new plate sealer. The plate was tapped to ensure mixing and incubated at 37°C in the dark. • 9. After 30 min, when an apparent gradient appeared in the standard wells, the reaction was terminated by adding 50 µL of Stop Solution to each well. • 10. The optical density (OD value) of each well was immediately read using a micro-plate reader set to 450 nm (with reference set to 570nm).

- 11. The triplicate readings for each standard were averaged and the average zero standard optical density subtracted. A standard curve was generated by plotting the normalized OD value for each standard on the y-axis against the concentration on the x-axis using Excel. A line of best fit through the points on the graph was used to generate the equation x = (y+0.0048) / 0.0249.
- 12. The equation x = (y+0.0048) / 0.0249 was used to calculate Cys-C concentrations of the samples based on their normalized average OD values.

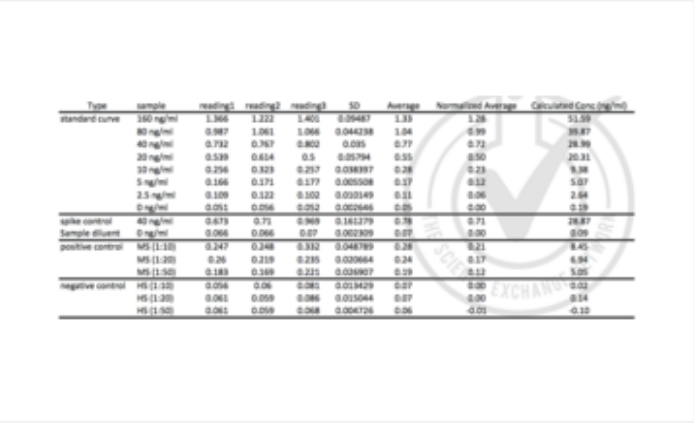
Experimental Notes:	- No challenges noted.

Images for Validation report #029704



Validation image no. 1 for Cystatin C (CST3) ELISA Kit (ABIN367853)

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings (excluding readings from 40, 80 and160 ng/ml) plotted for standard curve samples. Linear range is between 0 and 20 ng/ml.



Type	sample	reading1	reading2	reading3	SD	Average	Normalized Average	Calculated Conc (ng/ml)
standard curve	160 ng/ml	1.366	1.222	1.401	0.09487	1.33	1.28	51.59
	80 ng/ml	0.987	1.061	1.066	0.044238	1.04	0.99	39.87
	40 ng/ml	0.732	0.767	0.802	0.035	0.77	0.72	28.99
	20 ng/ml	0.539	0.614	0.5	0.05794	0.55	0.50	20.31
	10 ng/ml	0.256	0.323	0.257	0.038397	0.28	0.23	9.38
	5 ng/ml	0.166	0.171	0.177	0.005508	0.17	0.12	5.07
	2.5 ng/ml	0.109	0.122	0.102	0.010149	0.11	0.06	2.64
	0 ng/ml	0.051	0.056	0.052	0.002646	0.05	0.00	0.19
spike control	40 ng/ml	0.673	0.71	0.969	0.161279	0.78	0.71	28.87
Sample diluent	0 ng/ml	0.066	0.066	0.07	0.002309	0.07	0.00	0.09
positive control	NHS (1:10)	0.247	0.248	0.332	0.048789	0.28	0.21	8.45
	NHS (1:20)	0.26	0.219	0.235	0.020664	0.24	0.17	6.94
	NHS (1:50)	0.183	0.169	0.221	0.026907	0.19	0.12	5.05
negative control	HS (1:10)	0.056	0.06	0.081	0.013429	0.07	0.00	0.02
	HS (1:20)	0.061	0.059	0.086	0.015044	0.07	0.00	0.14
	HS (1:50)	0.061	0.059	0.068	0.004726	0.06	-0.01	-0.10

Validation image no. 2 for Cystatin C (CST3) ELISA Kit (ABIN367853)

Table 1: ELISA. Cys-1 is present in the positive control sample (Normal mouse serum) and absent from the negative control (Normal horse serum) sample. Spike control indicates no interference in absorbance reading from the Sample diluent (included in the kit) used to dilute the positive and negative control samples. Table of absorbance readings (OD 450 nm) is shown for standard curve, spike controls and unknown positive (normal mouse serum) and negative (normal horse serum) control samples. Value for Average Reading is derived from the average of three readings (OD 450nm). The Average Reading for 0ng/ml Standard was subtracted from all Average Readings of other Standards to yield normalized Average Absorbance

values for Standards. The Average Reading for Sample Dilution buffer was subtracted from Average Readings of spike control, mouse serum and horse serum to yield normalized Average Absorbance values for these samples. Standard deviation is included for all samples. An equation x = (y+0.0048) / 0.0249 was generated from the standard curve and used to calculate Cys-C concentrations shown in the Table. Undiluted Cys-C concentration in normal mouse serum was calculated from data obtained in 1:50 diluted mouse serum (recommended dilution factor from the instruction of the kit).