ab283970 – Human Cystatin S ELISA Kit

For the quantitative measurement of Cystatin S (CST4) in Human saliva and cell culture supernatant samples.
For research use only - not intended for diagnostic use.

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the Standard, SP Conjugate and Biotinylated Antibody which should be stored at -20°C.

Materials Supplied

Item	Quantity	Storage Condition
Human Cystatin S Microplate	96 wells	4°C
100X Streptavidin-Peroxidase (SP) Conjugate	80 µL	-20°C
10X Diluent M Concentrate	30 mL	4°C
1X Standard Diluent	2 mL	4°C
20X Wash Buffer Concentrate	2 x 30 mL	4°C
Chromogen Substrate	7 mL	4°C
Cystatin S Standard	1 vial	-20°C
Sealing Tapes	3 units	4°C
Stop Solution	11 mL	4°C
50X Biotinylated Human Cystatin S Antibody	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 nm
Pipettes (1-20 µl, 20-200 µl, 200-1000 µl, and multiple channel)
Deionized or distilled reagent grade water

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
Prepare only as much reagent as is needed on the day of the experiment.
If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved, and thoroughly rinse the bottles to remove any precipitate.
Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent M Concentrate: Dilute the Diluent M Concentrate 10-fold with reagent grade water to produce a 1X solution. Store for up to 30 days at 4°C.

50X Biotinylated Human Cystatin S Antibody: Spin down the antibody briefly and dilute the desired amount of the antibody 50-fold with 1X Diluent M to produce a 1X solution. The undiluted antibody should be stored at -20°C.

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1X solution.

100X SP Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 100-fold with Diluent M to produce a 1X solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

Always prepare a fresh set of standards for every use.
Prepare serially diluted standards immediately prior to use.
Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.
The following section describes the preparation of a standard curve for duplicate measurements (recommended).
Reconstitution of the CST4 Standard vial to prepare a 4000 pg/mL Standard Stock Solution.

- First consult the CST4 Standard vial to determine the mass of protein in the vial.
- Calculate the appropriate volume of **Standard Diluent** to add when resuspending the CST4 Standard vial to produce a 4000 pg/mL CST4 Standard Stock Solution by using the following equation:
    - C_S = Starting mass of CST4 Standard (see vial label) (pg)
    - C_F = The 4000 pg/mL CST4 Standard Stock Solution final required concentration
    - V_D = Required volume of Standard Diluent for reconstitution (µL)
    - Calculate total required volume Standard Diluent for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example: **Δ Note: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided**.
CS = 2000 pg of CST4 Standard in vial
CF = 4000 pg/mL CST4 Standard Stock Solution final concentration
VD = Required volume of Standard Diluent M for reconstitution (2000 pg / 4000 pg/mL) x 1,000 = 500 µL

- Reconstitute the CST4 Standard vial by adding the appropriate calculated amount VD of Standard Diluent to the vial to generate the 4000 pg/mL CST4 Standard Stock Solution. Mix gently and thoroughly.
- Allow the reconstituted 4000 pg/mL CST4 Standard Stock Solution to sit for 10 minutes with gentle agitation prior to making subsequent dilutions
- Label eight tubes #1 – 8.
- Add 360 µL of **1X Diluent M** to tube #1, and 120 µL of **1X Diluent M** to tube #2 – 8.
- To prepare Standard #1, add 120 µL of the Standard Stock Solution into tube #1 and mix gently.
- To prepare Standard #2, add 120 µL of the Standard #1 into tube #2 and mix gently
- Using the table below as a guide, prepare subsequent serial dilutions. Tube #8 1X Diluent M serves as the zero standard (0 pg/mL).

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	CST4 (pg/mL)
1	120 µL Standard Stock Solution (4000 pg/ml)	360 µL	1000
2	120 µL Standard #1	120 µL	500
3	120 µL Standard #2	120 µL	250
4	120 µL Standard #3	120 µL	125
5	120 µL Standard #4	120 µL	62.5
6	120 µL Standard #5	120 µL	31.25
7	120 µL Standard #6	120 µL	15.625
8	-	120 µL	0.0

Sample Preparation

Saliva

Collect saliva using sample tube.
Centrifuge samples at 800 x g for 10 minutes.
An 80000-fold sample dilution is suggested into 1X Diluent M; however, user should determine optimal dilution factor depending on application needs, within the range 1:5000-1:500000.
The undiluted samples can be stored at -20°C or below for up to 3 months.
Avoid repeated freeze-thaw cycles.

Cell Culture Supernatants

Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris and collect supernatant. If required, the samples can be diluted into 1X Diluent M; user should determine optimal dilution factor depending on application needs.
Samples can be stored at -80°C.
Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C for up to 30 days in a vacuum desiccator.
For each assay performed, a minimum of two wells must be used as the zero control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all standards, controls and samples in duplicate

1. Add 50 µL of Human CST4 Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
2. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; tap 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; tap 4-5 times on absorbent material to completely remove the liquid.
3. Add 50 µL of Biotinylated Human CST4 Antibody to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 1 hour.
4. Wash the microplate as described above.
5. Add 50 µL of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance
6. Wash the microplate as described above.
7. Add 50 µL of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate for 25 minutes or until the optimal blue color density develops.
8. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
9. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

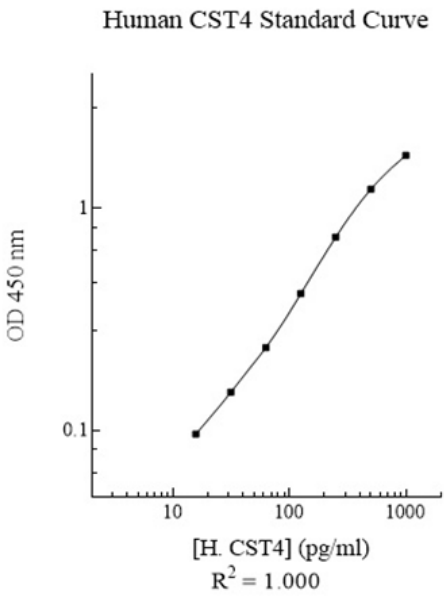
TYPICAL DATA

Typical data provided for demonstration purposes only.

Standard #	ng/mL	OD	Average OD
1	1.000	1.760 1.682	1.721
2	0.500	1.184 1.238	1.211
3	0.250	0.750 0.730	0.740
4	0.125	0.405 0.417	0.411
5	0.063	0.245 0.225	0.235
6	0.031	0.146 0.150	0.148
7	0.016	0.100 0.092	0.096
8	0.0	0.045 0.043	0.044

STANDARD CURVE

- The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.



PERFORMANCE CHARACTERISTICS

1. The minimum detectable dose of human CST4 as calculated by 2SD from the mean of a zero standard was established to be 7.2 pg/mL.
2. Intra-assay precision was determined by testing three samples twenty times in one assay.
3. Inter-assay precision was determined by testing three samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	4.7	9.5

SPIKING RECOVERY

Recovery was determined by spiking one serum and one saliva sample with different CST4 concentrations.

Sample	Unspiked Sample (pg/ml)	Spiking Value (pg/ml)	Expected	Observed	Recovery (%)
Serum	**338.007**	463.059	801.006	831.048	104
		122.862	460.869	453.160	98
		35.710	373.717	342.793	92
Saliva	**153.639**	463.059	616.698	639.002	104
		122.862	276.501	270.999	98
		35.710	189.349	192.484	102
Average Recovery (%)					**100**

LINEARITY

Saliva samples were serially diluted to test for linearity.

Average Percentage of Expected Value (%)	
Sample Dilution	**Saliva**
40000x	98%
80000x	96%
160000x	105%

CROSS REACTIVITY

Species	Cross-Reactivity (%)
CST1	10%

- No significant cross-reactivity observed with CSTA, CSTB, CST3, CST5, CST6, CST7, CST9, CST11, fetuin-A, kininogen (HMW), and kallikrein-2.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

For all technical or commercial enquiries please go to:
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