

ab201281 – Rat Cystatin C SimpleStep ELISA® Kit

For the quantitative measurement of Cystatin C in rat serum, plasma (citrate), plasma (EDTA), cell culture supernatant, and tissue extract.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab201281

Storage and Stability: Store kit at 2-8°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Standard Preparation and Reagent preparation sections.

Materials Supplied

Item	Quantity 1 x 96 tests	Storage Condition
Rat Cystatin C Capture Antibody 10X	600 µL	+4°C
Rat Cystatin C Detector Antibody 10X	600 µL	+4°C
Rat Cystatin C Lyophilized Recombinant Protein	2 Vials	+4°C
Antibody Diluent CP2	6 mL	+4°C
Cell Extraction Buffer PTR 5X	10 mL	+4°C
Cell Extraction Enhancer Solution 50X	1 mL	+4°C
Sample Diluent NS	50 mL	+4°C
Wash Buffer PT 10X	20 mL	+4°C
TMB Development Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
SimpleStep Pre-Coated 96-Well Microplate	96 wells	+4°C
Plate Seal	1	+4°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 or 600 nm.

Method for determining protein concentration (BCA assay recommended)

Deionized water.

Multi- and single-channel pipettes.

Tubes for standard dilution.

Plate shaker for all incubation steps.

Optional: Phenylmethylsulfonyl Fluoride (PMSF) (or other protease inhibitors).

Reagent Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells. The sample volumes below are sufficient for 48 wells (6 x 8-well strips); adjust volumes as needed for the number of strips in your experiment.

Prepare only as much reagent as is needed on the day of the experiment. Capture and Detector Antibodies have only been tested for stability in the provided 10X formulations.

The provided Cell Extraction Enhancer Solution 50X may precipitate when stored at + 4°C. To dissolve, warm briefly at + 37°C and mix gently. The Cell Extraction Enhancer Solution 50X can be stored at room temperature to avoid precipitation.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 100 mL 1X Wash Buffer PT combine 10 mL Wash Buffer PT 10X with 90 mL deionized water. Mix thoroughly and gently.

1X Cell Extraction Buffer PTR (For cell and tissue extracts only): Prepare 1X Cell Extraction Buffer PTR by diluting Cell Extraction Buffer PTR 5X and 50X Cell Extraction Enhancer Solution to 1X with deionized water. To make 10 mL 1X Cell Extraction Buffer PTR combine 7.8 mL deionized water, 2 mL Cell Extraction Buffer PTR 5X and 200 µL Cell Extraction Enhancer Solution 50X. Mix thoroughly and gently. If required protease inhibitors can be added.

10X Capture Antibody: To reconstitute the lyophilized capture antibody, centrifuge at 10,000 g for 2 minutes. Add 660 µL of Sample Diluent NS and gently mix on a rotator for 20 minutes at room temperature. Unused reconstituted antibody can be stored frozen at -20°C. Avoid repeated freeze-thaw cycles.

Antibody Cocktail: Prepare Antibody Cocktail by diluting the capture and detector antibodies in Antibody Diluent CP2. To make 3 mL of the Antibody Cocktail combine 300 µL 10X Capture Antibody and 300 µL 10X Detector Antibody with 2.4 mL Antibody Diluent CP2. Mix thoroughly and gently.

Standard Preparation

Always prepare a fresh set of standards for every use. Discard working standard dilutions after use as they do not store well. The following section describes the preparation of a standard curve for duplicate measurements (recommended).

1. Reconstitute the Cystatin C standard sample by adding the volume indicated on the protein vial label. For **serum, plasma and cell culture supernatant samples measurements**, use Sample Diluent NS. For **tissue extract samples measurements**, use 1X Cell Extraction Buffer PTR. Hold at room temperature for 10 minutes. Mix thoroughly and gently. This is the 7,500 pg/mL **Stock Standard** Solution.
2. Label eight tubes, Standards 1– 6.
3. Use the same Sample Diluent as used to resuspend the Stock Standard to prepare the standard curve. Add 240 µL of Sample Diluent into tube number 1 and 150 µL of Sample Diluent into numbers 2-6.
4. Use the **Stock Standard** to prepare the following dilution series. Standard #8 contains no protein and is the Blank control:

Standard #	Dilution Sample	Volume to Dilute (µL)	Volume of Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Stock Standard	60	240	7500	1500
2	Standard#1	150	150	1500	750
3	Standard#2	150	150	750	375
4	Standard#3	150	150	375	188
5	Standard#4	150	150	188	93.8
6	Blank Control	150	150	0	0

Sample Preparation

Typical Sample Dynamic Range	
Sample Type	Range
Rat Serum	0.003 - 0.2%
Rat Plasma - Citrate	0.006 - 0.4%
Rat Plasma - EDTA	0.006 - 0.4%
Stimulated Spleenocyte Supernatant	0.391 – 25%
Unstimulated Spleenocyte Supernatant	0.391 – 50%
Rat Heart Extract	3.91 - 125 µg/mL
Rat Brain Extract	0.488 - 15.6 µg/mL

Serum Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2,000 x g for 10 minutes and collect serum. Dilute samples at least 1:500 into Sample Diluent NS and assay. Store un-diluted serum at -20°C or below. Avoid repeated freeze-thaw cycles.

Plasma Collect plasma using citrate or EDTA. Dilute samples at least 1:250 into Sample Diluent NS and assay. Store un-diluted plasma samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Note: This kit is incompatible with plasma heparin samples.

Cell Culture Supernatants Centrifuge cell culture media at 2,000 x g for 10 minutes to remove debris. Collect supernatants. Dilute samples at least 1:2 into Sample Diluent NS and assay. Store un-diluted samples at -20°C or below. Avoid repeated freeze-thaw cycles.

Preparation of extracts from tissue homogenates Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly.

Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and

store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

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 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).

Differences in well absorbance or “edge effects” have not been observed with this assay.

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. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, reseal and return to 4°C storage.
3. Add 50 µL of all sample or standard to appropriate wells.
4. Add 50 µL of the Antibody Cocktail to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm.
6. Wash each well with 3 x 350 µL 1X Wash Buffer PT. Wash by aspirating or decanting from wells then dispensing 350 µL 1X Wash Buffer PT into each well. Wash Buffer PT should remain in wells for at least 10 seconds. Complete removal of liquid at each step is essential for good performance. After the last wash invert the plate and tap gently against clean paper towels to remove excess liquid.
7. Add 100 µL of TMB Development Solution to each well and incubate for 10 minutes in the dark on a plate shaker set to 400 rpm.

Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.

Note: The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.

8. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.
9. Alternative to 7 – 8: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	up to 20 min
Interval:	20 sec - 1 min

Shaking:	Shake between readings
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Note that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 μ L Stop Solution to each well and recording the OD at 450 nm.

Download our **ELISA guide for technical hints, results, calculation, and troubleshooting tips:**

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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Additional information

ASSAY SPECIFICITY

This kit is designed for the quantification of rat Cystatin C.

SPECIES REACTIVITY

This kit recognizes rat Cystatin C protein.

Other species reactivity was determined by measuring 0.1% (1:1,000) serum samples of various species, interpolating the protein concentrations from the rat standard curve, and expressing the interpolated concentrations as a percentage of the protein concentration in rat serum assayed at the same dilution.

Reactivity < 3% was determined for the following species:

- Rabbit
- Dog
- Cow
- Pig

Serum showing reactivity:

Species	% Cross-reactivity
Mouse	100

CALCULATION

- Calculate the average absorbance value for the blank control (zero) standards. Subtract the average blank control standard absorbance value from all other absorbance values.
- Create a standard curve by plotting the average blank control subtracted absorbance value for each standard concentration (y-axis) against the target protein concentration (x-axis) of the standard. Use graphing software to draw the best smooth curve through these points to construct the standard curve.
 Δ Note: Most microplate reader software or graphing software will plot these values and fit a curve to the data. A four-parameter curve fit (4PL) is often the best choice; however, other algorithms (e.g., linear, semi-log, log/log, 4-parameter logistic) can also be tested to determine if it provides a better curve fit to the standard values.
- Determine the concentration of the target protein in the sample by interpolating the blank control subtracted absorbance values against the standard curve. Multiply the resulting value by the appropriate sample dilution factor, if used, to obtain the concentration of target protein in the sample.
- Samples generating absorbance values greater than that of the highest standard should be further diluted and reanalyzed. Similarly, samples which measure at absorbance values less than that of the lowest standard should be retested in a less dilute form.

TYPICAL DATA

Typical standard curve – data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.195	0.190	0.192
93.8	0.323	0.317	0.320
188	0.459	0.448	0.454
375	0.685	0.669	0.677
750	1.248	1.216	1.232
1,500	2.345	2.348	2.346

Table 1. Example of rat Cystatin C standard curve in Sample Diluent NS. The Cystatin C standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

Standard Curve Measurements			
Concentration (pg/mL)	O.D. 450 nm		Mean O.D.
	1	2	
0	0.107	0.104	0.105
93.8	0.191	0.186	0.189
188	0.269	0.269	0.269
375	0.414	0.420	0.417
750	0.755	0.748	0.752
1,500	1.495	1.490	1.492

Table 2. Example of rat Cystatin C standard curve in 1X Cell Extraction Buffer PTR. The Cystatin C standard curve was prepared as described in the Standard Preparation section. The table shows raw data values.

TYPICAL SAMPLE VALUES

Sensitivity:

The minimal detectable dose (MDD) was determined by calculating the mean of zero standard replicates and adding 2 standard deviations then extrapolating the corresponding concentration.

Sample Diluent Buffer	N=	Minimal Detectable Dose
Sample Diluent NS	32	25.58 pg/mL
1X Cell Extraction Buffer PTR	32	28.8 pg/mL

Recovery

Three concentrations of Cystatin C were spiked into the indicated biological matrix to evaluate signal recovery in the working range of the assay.

Sample Type	Average % Recovery	Range (%)
0.025% Rat Serum	113	111 - 114
0.05% Rat Plasma - Citrate	112	109 - 116
0.05% Rat Plasma - EDTA	115	108 - 119
50% Cell Culture Media	110	107 - 116

Linearity of Dilution

Linearity of dilution is determined based on interpolated values from the standard curve. Linearity of dilution defines a sample concentration interval in which interpolated target concentrations are directly proportional to sample dilution.

Native Cystatin C was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in Sample Diluent NS.

Dilution Factor	Interpolated value	0.2% Rat Serum	0.4% Rat Plasma (Citrate)	0.4% Rat Plasma (EDTA)	25% Rat Stimulated Spleenocyte Supernatant	50% Rat Unstimulated Spleenocyte Supernatant
Undiluted	pg/mL	2,472	2,711	2,431	2,600	2,771
	% Expected	100	100	100	100	100
2	pg/mL	1,213	1,417	1,176	1,287	1,564
	% Expected	98	105	97	99	113
4	pg/mL	555	649	556	611	740
	% Expected	90	96	91	94	107
8	pg/mL	284	318	272	300	355
	% Expected	92	94	89	92	102
16	pg/mL	140	154	133	145	176
	% Expected	91	91	88	89	102

Native Cystatin C was measured in the following biological samples in a 2-fold dilution series. Sample dilutions are made in 1X Cell Extraction Buffer PTR.

Dilution Factor	Interpolated value	125 µg/mL	15.6 µg/mL
		Rat Heart Extract	Rat Brain Extract
Undiluted	pg/mL	2,877	1,759
	% Expected	100	100
2	pg/mL	1,443	842
	% Expected	100	96
4	pg/mL	675	407
	% Expected	94	93
8	pg/mL	331	202
	% Expected	92	92
16	pg/mL	145	98.4
	% Expected	81	90

Precision

Mean coefficient of variations of interpolated values of Cystatin C from 3 concentrations of Cystatin C within the working range of the assay.

	Intra-assay	Inter-assay
N=	8	3
CV (%)	2.95	3.52

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus

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