

Rabbit Cystatin-C, Cys-C ELISA Kit

96 Tests

Operating instruction

FOR RESEARCH USE ONLY!

Alternative name

Rabbit Cys-C ELISA KIT ,Rabbit CysC ELISA KIT ,Rabbit Cystatin C ELISA KIT ,Rabbit ARMD11 ELISA KIT ,Rabbit HEL-S-2 ELISA KIT ,Rabbit cystatin 3 ELISA KIT ,Rabbit cystatin-3 ELISA KIT ,Rabbit epididymis secretory protein Li 2 ELISA KIT ,Rabbit gamma-trace ELISA KIT ,Rabbit neuroendocrine basic polypeptide ELISA KIT ,Rabbit post-gamma-globulin ELISA KIT ,Rabbit Cystatin-C ELISA KIT ,Rabbit Cystatin-3 ELISA KIT ,Rabbit Gamma-trace ELISA KIT

Intended use

Rabbit Cys-C ELISA KIT allows for the in vitro quantitative determination of rabbit Cystatin-C concentrations in serum, Plasma, tissue homogenates and Cell culture supernates and Other biological fluids.

Test principle

The microtiter plate provided in **Rabbit Cys-C ELISA KIT** has been pre-coated with an antibody specific to Cystatin-C. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for Cystatin-C and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain Cystatin-C, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm \pm 2 nm. The concentration of Cystatin-C in the samples is then determined by comparing the O.D. of the samples to the standard curve

Materials and components

Reagent	Quantity
Assay plate	1
Standard	2
Sample Diluent	1 \times 20ml
Assay Diluent A	1 \times 10ml
Assay Diluent B	1 \times 10ml
Detection Reagent A	1 \times 120 μ l
Detection Reagent B	1 \times 120 μ l
Wash Buffer(25 x concentrate)	1 \times 30ml
Substrate	1 \times 10ml
Stop Solution	1 \times 10ml
Plate sealer for 96 wells	5
Instruction	1

Other supplies required

Microplate reader.

Pipettes and pipette tips.

EP tube

Deionized or distilled water.

Storage of the kits

The **Assay Plate, Standard, Detection Reagent A and Detection Reagent B** should be stored at -20°C upon being received. After receiving the kit, **Substrate should be always stored at 4°C** . Other reagents are kept according to the labels on vials. But for long term storage, please keep the whole kit at -20°C . The unused strips should be kept in a sealed bag with the desiccant provided to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit (six months from the date of manufacture). Opened test kits will remain stable until the expiring date shown, provided it is stored as prescribed above.

Sample collection and storage

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately $1000 \times g$. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C .

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at $1000 \times g$ at 2°C - 8°C within 30 minutes of collection. Store samples at -20°C or -80°C . Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at $\leq -20^{\circ}\text{C}$. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at $5000 \times g$. Remove the supernate and assay immediately or aliquot and store at $\leq -20^{\circ}\text{C}$.

Cell culture supernates and Other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C . Avoid repeated freeze-thaw cycles.

Note:

1. Samples to be used within 5 days may be stored at $2-8^{\circ}\text{C}$, otherwise samples must be stored at -20°C (1 month) or -80°C (2 months) to avoid loss of bioactivity and contamination.
2. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals.
3. Influenced by the factors including cell viability, cell number and also sampling time, samples from cell culture supernatant may not be detected by the kit.
4. Sample hemolysis will influence the result, so hemolytic specimen can not be detected.
5. When performing the assay slowly bring samples to room temperature.
6. Do not use heat-treated specimens.

Limitations of the procedure

1. Nova Lifetech. is only responsible for the kit itself, but not for the samples.

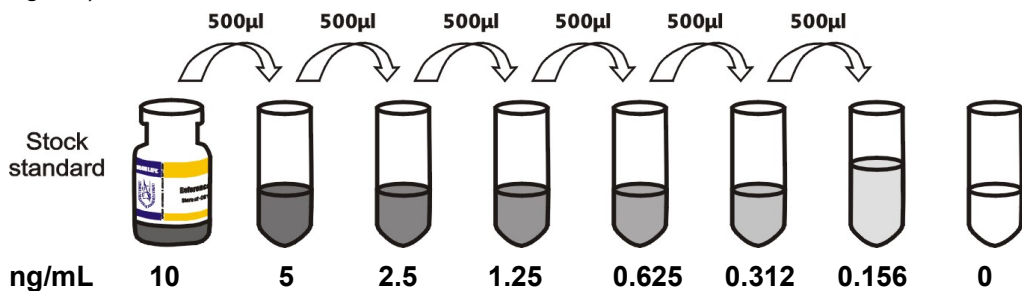
consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2. The kit should not be used beyond the expiration date on the kit label.
3. Do not mix or substitute reagents with those from other lots or sources.
4. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

Reagent preparation

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer.

Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent. This reconstitution produces a stock solution of 10 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (10 ng/mL). The Sample Diluent serves as the zero standard (0 ng/mL).



Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A(1;100) and B (1:100), respectively.

Assay procedure

Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37°C directly.). **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.** Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4°C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. 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.

1. Add 100 µl of **Standard**, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37°C.
2. Remove the liquid of each well, don't wash. Add 100 µl of **Detection Reagent A** working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at

37°C. Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

3. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µl) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. and let it sit for 1~2 minutes. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
4. Add 100 µl of **Detection Reagent B** working solution to each well. Cover with a new Plate sealer. Incubate for 1 hour at 37°C.
5. Repeat the aspiration/wash process for 5 times as conducted in step 3..
6. Add 90 µl of **Substrate Solution** to each well. Cover with a new Plate sealer. Incubate within **15-30 minutes** at 37°C. Protect from light.
7. Add 50 µl of **Stop Solution** to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density of each well at once, using a microplate reader set to 450 nm.

Note:

1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption.
2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. **The reconstituted Standards Detection Reagent A and B can be used only once.** This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10µl for once pipetting.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, **DO NOT** let the strips DRY at any time during the assay.
4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes.
5. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
7. Duplication of all standards and specimens, although not required, is recommended.
8. Substrate Solution is easily contaminated. Please protect it from light.
9. The PDF version of manual is only for reference, subject to the instruction shipping