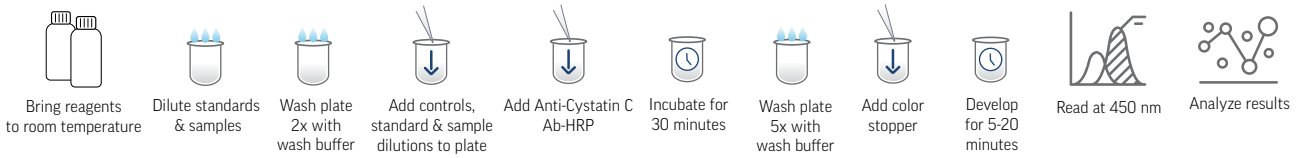
MOUSE CYSTATIN C ELISA



Description

Mouse Cystatin C ELISA is a direct antibody capture competitive assay for the determination of cystatin C in biological samples. It is for research purposes and is not intended for diagnostic use.

The assay uses recombinant mouse cystatin C (Ala21-Ala140) as standard and a polyclonal antibody raised against recombinant cystatin C. This antibody does not react with mouse albumin or mouse IgG. Native cystatin C samples produced dose-response curves that were colinear with those made with Ethos Biosciences kit standards.

Kit Contents

1. 1 x 96-well Mouse Cystatin C Assay Plate
2. 1 vial Mouse Cystatin C Standard
3. 2 x 12 mL NHECas (Diluent)
4. 1 x 6 mL Anti-Mouse Cystatin C Ab-HRP
5. 1 x 12 mL TMB Developer
6. 1 x 12 mL Acid Stopper

Note: Items 2 through 4 contain Proclin® 300 (isothiazolinones) as preservative. The Cystatin C Assay Plate is precoated, blocked, and dried. The reagents are supplied in ready-to-use form. Color Stopper contains 2.0 N Sulfuric Acid.

SDS is available at the Ethos Biosciences' website (www.ethosbiosciences.com). Store any unused reagents and strips (in sealed pouch with included desiccant pack) at 4˚C for future use. Do not use this kit beyond its expiration date.

Other Materials Required but Not Provided

- Wash buffer such as Ethos TEA Wash Buffer
- Microplate reader equipped to measure absorbance at 450 nm is required
- Adjustable pipettes and pipette tips
- Multi-channel pipettor(s) and tips for dispensing 50 µL and 100 µL volumes
- Tubes for preparing dilutions, i.e. microfuge tubes
- Wash bottle or automated microplate washer
- Incubation chamber: a closed humid container, for example a plastic food storage container with a tight-fitting lid containing a water moistened paper towel.

Specimen Collection and Storage

Urine: Collect urine samples without preservative and centrifuge to remove particulates. Aliquot and store at ≤-70˚C. Avoid repeated freeze-thaw cycles.
Serum: Allow blood to clot, centrifuge 15 minutes at 1000 x g, and recover the serum. Assay immediately or aliquot and store at ≤-70˚C. Avoid repeated freeze-thaw cycles.
Saliva: Collect saliva using appropriate collection device. Assay immediately or aliquot and store samples at ≤-70˚C. Avoid repeated freeze-thaw cycles.
Tissue Culture Supernatant/ Cell Lysate: Centrifuge all samples to remove debris. Assay immediately or store samples at ≤-70˚C. Avoid repeated freeze-thaw cycles.

Limitations

FOR RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES.

- It is the responsibility of the investigator to determine if the presence of experimental compounds or their metabolites in the sample that may affect the assay results.
- Gross hemolysis, lipemia, or microbiological contamination of samples may affect assay results.
- Do not mix or substitute reagents with those from other kits or sources.
- Do not use the kit beyond the expiration date on the kit label.

Assay Procedure

This procedure describes the controls, standard and sample dilutions, and their addition to the plate. Standard and sample dilutions are assayed in duplicate wells which allows analysis of up to 40 samples.

Allow reagents and samples to come to room temperature before running the assay. (Note: The assay performs better when room temperature is between 20˚C and 25˚C.)

Allow frozen samples to thaw at room temperature, and gently mix to assure homogeneity. Leave the samples undisturbed for 30-60 minutes to allow particulates to settle out.

Standard Dilutions: This procedure describes the serial dilution of Cystatin C Standard.

1. Label the tubes numbers 1-7.

2. Add 120 µL of NHECas Diluent per tube. Transfer 120 µL of Cystatin C Standard Stock to tube 1; this is a 1:2 dilution of the standard.
3. Mix contents by aspirating and expelling the fluids 5 times.
4. Transfer 120 µL of solution from tube 1 to tube 2.
5. Mix as before.
6. Continue this procedure through tube number 7.
7. Tubes 1-7 now contain dilutions representing 500, 250, 125, 62.5, 31.25, 15.62, 7.81 ng cystatin C/mL.

Preparation of Sample Dilutions: Samples must be diluted to fall into the range of the assay. Following are suggested dilutions, but additional dilutions may be necessary for initial studies.

1. For urine samples, a starting dilution of 1:5 is suggested.
2. For serum samples, a 1:20 dilution is suggested.
3. It is recommended that sample dilutions be performed in separate tubes before adding to assay plate.

Label the Plate: Label the tabs on the strips with an indelible marker 1-12. This will allow reconstruction of the plate should strips fall out during the washing procedures.

Wash the Plate 2 Times: Use TEA Wash buffer

1. Fill the wells with TEA Wash Buffer.
2. Remove the wash buffer by flipping the plate out into the sink or by aspirating the fluid with a plate washer.
3. Repeat.
4. Blot excess fluids from plate by inverting on a clean paper towel.

Addition of Controls, Cystatin C Standard Dilutions, and Samples to the plate: The plate design described here includes two controls: a negative control (no antibody conjugate) termed C0, and a positive one (no competitor) termed C1. These are placed in wells A1, and A2 respectively. All other wells receive either diluted standard or diluted sample

1. Add 100 µL NHECas (Diluent) from the stock bottle to well A1. This is the negative control "C0" and will be used to standardize or "blank" the microplate reader.
2. Add 50 µL Diluent to well A2. This is the positive control "C1" and is a qualitative indicator of assay performance.
3. With a fresh tip, transfer 50 µl aliquots of Cystatin C Standard Dilution Tube 1 (1:2 dilution of stock) to wells B1 and B2.
4. With a fresh tip, transfer 50 µL aliquots Cystatin C Standard Dilution Tube 2, and to wells C1 and C2.
5. Continue transferring diluted standard to the plate in this fashion, i.e. in order through H1 and H2, taking care to use a fresh tip for each new dilution.
6. Using a new tip, add 50 µL aliquots of Diluted Sample to wells A3 and A4.
7. Continue adding diluted samples to the plate, taking care to change the tip for each one.
8. The plate now contains controls, standard dilutions, and diluted experimental samples in duplicate.

Primary Incubation: Reaction with Anti-Cystatin C Antibody-HRP conjugate.

1. Well A1 is the negative control. Do NOT add conjugate to this well.
2. Add 50 µL of conjugate to the rest of the plate: to well A2 and to all remaining wells. A multichannel pipettor is recommended.
3. Cover and incubate the plate in a incubation chamber at room temperature for 30 minutes.

Wash the Plate 5 Times:

1. Flip plate out into the sink or aspirate off the reaction volume.
2. Fill the wells with TEA Wash Buffer (using a squirt bottle or a plate washer), and remove it by flipping it out into the sink or by aspiration. This constitutes a single wash cycle.
3. Wash the plate a total of five (5) wash cycles.
4. Invert the plate on a clean paper towel and tap gently to blot any adherent fluids. Do not allow plate to dry out.

Color Development:

1. Add 100 µL of Color Developer to each well.
2. Develop 5-20 minutes.
3. Add 100 µL of Color Stopper to each well. The color will change from blue to yellow.

Measure the OD at 450 nm: Use a plate reader to determine and record the absorbance of all wells at 450 nm, blanked against well A1.

Analysis: Color development in the assay is inversely proportional to the log of cystatin C concentration of the diluted standard or sample. The dose response for cystatin C may be sigmoidal in shape, in which case a logistics model may provide a better fit for the data.

Computer-based curve-fitting software program for a 4-parameter logistics model (4PL): fitting mean absorbance (y-axis) against the protein concentration (x-axis). The cystatin C concentration of the samples can then be interpolated from the standard curve. Multiply the concentration by the dilution factor to determine the undilute sample concentration.

Alternatively, prepare a spreadsheet entering appropriate data including standard dilution, concentration, log concentration, sample dilution, and absorbance data. Determine the mean for replicate wells.

Prepare a semi-logarithmic plot of the standard curve: Place the log [cystatin C] on the x-axis and the mean absorbance on the y-axis. The data that fall into the linear portion of the dose response curve constitute the usable portion of the assay. Subject these data to semi-logarithmic analysis to yield a mathematical model, of the form:

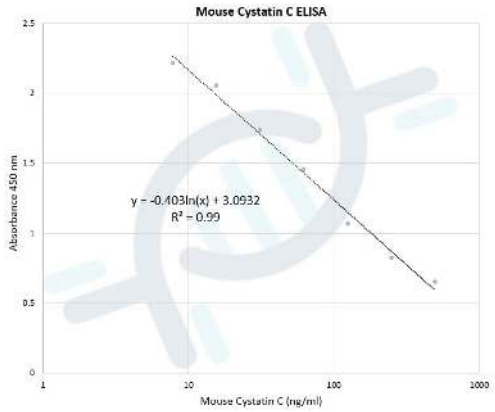
$A450 = (m)\ (\log10[\text{cystatin C}])+b$

which rearranges to:

$\log10\ [\text{cystatin C}] = (A450 - b)/m$

Cystatin C concentration is determined by taking the anti-log of the calculated values from this equation.

Multiply by the dilution factor of the sample to determine the concentration of undilute sample.



Quality Control

Record Keeping: It is good laboratory practice to record the lot numbers and dates of the kit components and reagents for each assay.

Sample Handling: The samples should be secured, processed, and stored as discussed above.

Dilutions: Dilute Standard and Samples carefully. For each standard and sample, a fresh tip should be used.

Template: Record the position of each standard or sample on a microplate template.

Troubleshooting

1. No color appears after adding Color Developer: One or more reagents may not have been added. Repeat assay. Be sure to store the kit appropriately. Do not use expired kits or reagents from other kits.
2. Color in wells too light: Longer incubation with Color Developer may be required. If the color is still too light after 20 minutes development, repeat the assay but increase the incubation with conjugate to 60 minutes.
3. Color in wells is too dark: Be sure to wash with the appropriate buffer. Be sure to blot dry after washing. Do not allow wells to dry out. Ensure that the standards and samples were added to the plate first followed by the primary antibody. Decrease the development time.
4. If color is dark and the standard dilutions fail to show the appropriate dose-response, Color Developer may have been contaminated with conjugate or the plate was poorly washed. The color developer should be clear to a very pale blue at room temperature. Be sure that all reagents, standards, and samples are at room temperature throughout the assay. Repeat the assay and take care in the pipetting and in the washing operations.
5. Color in sample well(s) is darker or lighter than lowest or highest concentrations of the standard curve. Change sample dilution protocol appropriately.
6. Poor agreement between duplicate wells: This is almost always due to pipetting error. Repeat the assay.
7. Microplate ELISAs are prone to edge effects wherein the outer rows and columns show a darker response than the inner ones. This effect may be minimized by incubating the plate in a closed humid container.

PRODUCT INFORMATION

CAT. #	DESCRIPTION
1043	Mouse Cystatin C ELISA

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TRADEMARKS