KAMIYA BIOMEDICAL COMPANY

Mouse Clusterin (CLS) ELISA

For the quantitative determination of clusterin in mouse biological samples

Cat. No. KT-682

For Research Use Only.

PRODUCT INFORMATION

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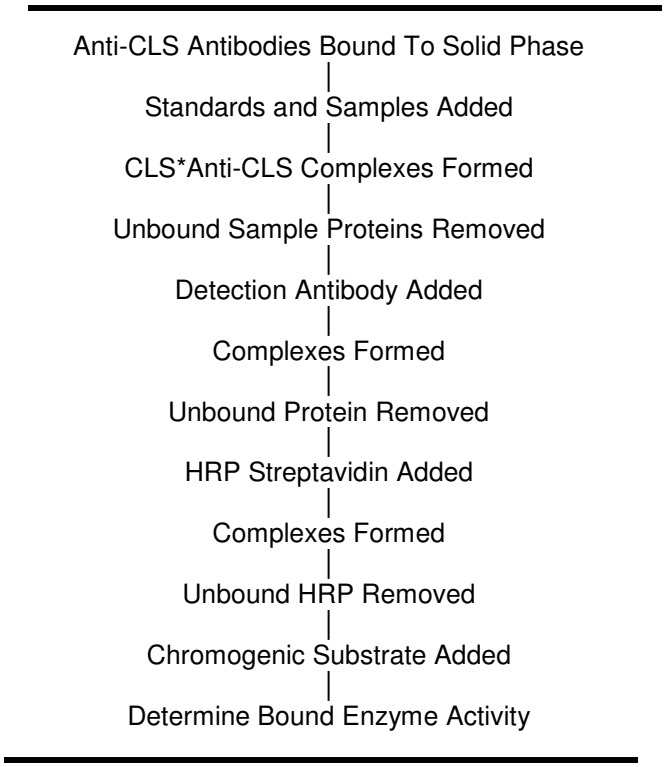
INTENDED USE
The Mouse Clusterin (CLS) ELISA is a highly sensitive two-site enzyme-linked immunoassay (ELISA) for the quantitative determination of CLS in mouse biological samples. For research use only.

PRINCIPLE
The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the CLS present in samples reacts with the anti-CLS antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, the Detection Antibody, biotin conjugated anti-CLS, is added and complexes are formed. Following a wash step, the horseradish peroxidase (HRP) conjugated Streptavidin is added and complexes are formed. After another washing step, the complexes are assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of CLS in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of CLS in the test sample. The quantity of CLS in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Figure 1.



COMPONENTS

1. DILUENT (Running Buffer)
One bottle containing 50 ml of a 1X diluent running buffer.

2. WASH SOLUTION CONCENTRATE
One bottle containing 50 ml of a 20X concentrated wash solution.

3. DETECTION ANTIBODY 100X
One vial containing 150 µL of affinity purified anti-Mouse Clusterin antibody conjugated with biotin in a stabilizing buffer.

4. HRP-STREPTAVIDIN 100X
One vial containing 150 µL of HRP conjugated streptavidin in a stabilizing buffer.

5. CHROMOGEN-SUBSTRATE SOLUTION
One vial containing 12 mL of 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

6. STOP SOLUTION
One vial containing 12 ml 0.3 M sulfuric acid.
WARNING: Avoid contact with skin.

7. ANTI-MOUSE CLUSTERIN ELISA MICRO PLATE
Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Mouse CLS.

8. MOUSE CLUSTERIN CALIBRATOR
One vial containing a lyophilized Mouse Clusterin calibrator.

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipettes (2 µL to 200 µL) for making and dispensing dilutions
- Test tubes
- Microplate washer/aspirator
- Distilled or de-ionized H_2O
- Microplate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

REAGENT PREPARATION

1. DILUENT
Ready to use as supplied.

2. WASH SOLUTION CONCENTRATE
The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35°C before dilution can dissolve crystals.

3. DETECTION ANTIBODY 100X
Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL detection antibody to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. HRP-STREPTAVIDIN 100X
Calculate the required amount of working conjugate solution for each microtiter plate test strip by adding 10 µL HPR-streptavidin to 990 µL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

5. CHROMOGEN-SUBSTRATE SOLUTION
Ready to use as supplied.

6. STOP SOLUTION
Ready to use as supplied.

7. ANTI-MOUSE CLUSTERIN ELISA MICRO PLATE
Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

8. MOUSE CLUSTERIN CALIBRATOR
Add 1.0 ml of distilled or de-ionized water to the Mouse Clusterin Calibrator and mix gently until dissolved. The calibrator is now at a concentration of 5.3 ug/ml **(the reconstituted calibrator should be aliquoted and frozen if future use is intended).** Mouse Clusterin standards need to be prepared immediately prior to use (see the following chart). Mix well between each step. Avoid foaming.

Standard	ng/ml	Volume added to → 1x Diluent	Volume of 1x Diluent
7	100	15 µl Mouse CLS Calibrator	780 µl
6	50	300 µl standard 7	300 µl
5	25	300 µl standard 6	300 µl
4	12.5	300 µl standard 5	300 µl
3	6.25	300 µl standard 4	300 µl
2	3.125	300 µl standard 3	300 µl
1	1.56	300 µl standard 2	300 µl
0	0		600 µl

STORAGE AND STABILITY
The expiration date for the package is stated on the box label.

1. DILUENT
The Diluent is stable until the expiration date and should be stored at 4°C.

2. WASH SOLUTION
The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25°C) or at 4°C.

3. DETECTION ANTIBODY 100X
Undiluted Biotin conjugated anti-Mouse Clusterin should be stored at 4°C and **diluted immediately prior to use**. The working conjugate solution is stable for up to 1 hour when stored in the dark.

4. HRP-STREPTAVIDIN 100X
Undiluted horseradish peroxidase conjugated streptavidin should be stored at 4°C and **diluted immediately prior to use**. The working conjugate solution is stable for up to 1 hour when stored in the dark.

5. CHROMOGEN-SUBSTRATE SOLUTION
The Substrate Solution should be stored at 4°C and is stable until the expiration date.

6. STOP SOLUTION
The Stop Solution should be stored at 4°C and is stable until the expiration date.

7. ANTI-MOUSE CLUSTERIN ELISA MICRO PLATE
Anti-Mouse CLS coated wells are stable until the expiration date, and should be stored at 4°C in sealed foil pouch with desiccant pack.

8. MOUSE CLUSTERIN CALIBRATOR
The lyophilized Mouse Clusterin calibrator should be stored at 4C or frozen until reconstituted. The reconstituted calibrator should be aliquoted out and stored frozen (Avoid multiple freeze-thaw cycles). The working standard solutions should be prepared immediately prior to use and are stable for up to 1 hour.

INDICATIONS OF INSTABILITY

If the test is performing correctly, the results observed with the calibrator solutions should be within 20% of the expected values.

SPECIMEN COLLECTION AND HANDLING

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20°C. Avoid repeated freeze-thaw cycles.

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds. No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

ASSAY PROTOCOL

Dilution of Samples

The assay for quantification of CLS in samples requires that each test sample be diluted before use. For a single step determination a dilution at 1:4,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the calibration curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

To prepare a 1:4,000 dilution of sample, transfer 2 µL of sample to 198 µL of 1X Diluent. This gives you a 1:100 dilution. Mix thoroughly. Then dilute this by transferring 10 µL to 390 µL of 1X Diluent. You now have a 1:4,000 dilution of your sample. Make sure to mix thoroughly at each stage.

Procedure

1. Bring all reagents to RT before use.

2. Pipette 100 µL of
   - Calibrator 0 (0.0 ng/mL) in duplicate
   - Calibrator 1 (1.56 ng/mL) in duplicate
   - Calibrator 2 (3.125 ng/mL) in duplicate
   - Calibrator 3 (6.25 ng/mL) in duplicate
   - Calibrator 4 (12.5 ng/mL) in duplicate
   - Calibrator 5 (25 ng/mL) in duplicate
   - Calibrator 6 (50 ng/mL) in duplicate
   - Calibrator 7 (100 ng/mL) in duplicate

3. Pipette 100 µL of diluted sample (in duplicate) into pre-designated wells.

4. Incubate the Microtiter Plate at 22°C (RT) for 2 hours. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with diluted Wash Solution, invert the plate and pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual Wash Solution. Repeat three times for a total of four washes.

7. Pipette 100 µL of appropriately diluted detection antibody to each well. Incubate at RT for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5 and 6.

9. Pipette 100 µL of appropriately diluted HRP-streptavidin to each well. Incubate at RT for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

10. Wash and blot the wells as described in Steps 5 and 6.

11. Pipette 100 µL of TMB Substrate Solution into each well.

12. Incubate in the dark at RT for precisely ten (10) minutes.

13. After ten minutes, add 100 µL of Stop Solution to each well.

14. Determine the absorbance at 450 nm of the contents of each well. Zero the plate reader to manufacture's specifications.

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

RESULTS

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the calibrators construct a calibration curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from calibration curve. Correct for sample dilution factor to arrive at CLS concentration in original sample.

QUALITY CONTROL

In accord with good laboratory practice, the assays for specific CLS require meticulous quality control. Each laboratory should use routine quality control procedures to establish inter- and intra-assay precision and performance characteristics.

LIMITATION OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.

2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or de-ionized water, and accuracy of reagent and sample pipetting, washing technique, incubation time or temperature.

FOR RESEARCH USE ONLY

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