Mouse LDL (Low Density Lipoprotein) CLIA Kit

NB-22-41703-1

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Cat# NB-22-41703-1 size: 96 wells

We highly recommended reading this manual thoroughly before using this kit.

Introduction

This CLIA kit is for quantitative determination of LDL in mouse serum, plasma, tissue homogenates, cell lysates, cell culture supernatant and other biological fluids.

Principle of The Assay

This CLIA kit uses the Sandwich-CLIA principle. The micro plate provided in this kit has been pre-coated with an antibody specific to mouse LDL. Standards or samples are added to the micro plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for mouse LDL and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain mouse LDL, biotinylated detection antibody and Avidin-HRP conjugate will show fluorescence. The relative light unit (RLU) value is measured by a chemiluminescent immunoassay analyser. The RLU value is positively associated with the concentration of mouse LDL. You can calculate the concentration of mouse LDL in the samples by comparing the RLU of the samples to the standard curve.

Sensitivity: 0.94 ng/mL

Detection Limit: 1.56 ~ 100 ng/mL

Materials Provided

1) Micro-CLIA Coated Plate 1 plate, 8×12

   Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20°C for up to 6 months. Reseal along entire edge of zip-seal.

2) Standard (Lyophilized) x 2

   Aliquot and store at ≤ -20 °C for up to 6 months. * Avoid repeated freeze-thaw cycles.

3) Concentrated Biotinylated Detection Antibody (100×) 1 ×120 µL

   May be stored for up to 6 months at -20°C. Protect from light.

4) Streptavidin-HRP Concentrated (100×) 1 ×120 µL

   May be stored for up to 6 months at -20°C. Protect from light.

5) Standard/Sample Diluent 20 mL

   May be stored for up to 6 months at 2-8°C.

6) Biotinylated Detection Antibody Diluent 14 mL

   May be stored for up to 6 months at 2-8°C.

7) Streptavidin-HRP Diluent 14 mL

May be stored for up to 6 months at 2-8°C.

8) Wash Buffer (25x) 30 mL

May be stored for up to 6 months at 2-8°C.

9) Substrate Reagent A 5 mL

May be stored for up to 6 months at 2-8°C. Protect Substrate from light.

10) Substrate Reagents B 5ml

May be stored for up to 6 months at 2-8°C. Protect Substrate from light.

11) Plate Sealers 5 Strips

Sample Collection and Storage

1. Cell Culture Supernatant

Centrifuge 1000xg for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles. If cell culture supernatant samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent.

2. Serum

Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000xg. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

3. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately, or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

4. Cell Lysates

Cells need to be lysed before assaying according to the following directions. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1000×g for 5 minutes (suspension cells can be collected by centrifugation directly). Wash cells 3 times in cold PBS. Resuspend cells in fresh PBS with concentration of 1 x 10^6 cells/mL. If it is necessary, the cells could be subjected to ultrasonication until the solution is clarified. Centrifuge at 1500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.. **Assay immediately or aliquot and store at ≤-20°C.**

5. Tissue homogenates

The preparation of tissue homogenates will vary depending upon tissue type. Tissues should be rinsed thoroughly in ice-cold PBS (00.01M, pH7.4) to remove excess blood, and weighed before homogenization. Mince the tissues to small pieces and homogenise them in fresh PBS (at 1:9, tissue:volume) with a glass homogeniser. The resulting suspension should be sonicated with an ultrasonic cell disrupter until the solution is clarified. Centrifuge the homogenates for 5 minutes at 10000×g and collect the supernatant. Assay immediately or aliquot and store at ≤-20°C.**Assay immediately or aliquot and store at ≤-20°C.**

6. Other biological fluids

Centrifuge samples for 20 minutes at 1000×g. Collect the supernatant and assay immediately or store samples in aliquot at -20°C or -80°C for later use. **Avoid repeated freeze/thaw cycles. Avoid haemolytic and hyperlipidaemia samples for serum and plasma.**

Dilution: Dilute samples at the appropriate multiple (recommend carrying out a pre-test to determine the dilution factor).

Note

Samples should be assayed within 7 days when stored at 2-8°C, otherwise samples must be divided up and stored at -20℃ (≤1 month) or -80℃ (≤3 months), avoiding freeze-thaw cycles. We recommend predicting the concentration before assaying. If the sample concentration is not within the range of the standard curve users should determine the optimal sample dilutions for their particular experiments. If the sample type is not included in this manual, a preliminary experiment is advised to verify the validity. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility of causing a deviation to the results. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.

Precautions

1. This kit is for RESEARCH USE ONLY.
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful. If contact made with skin, rinse with an excess amount of tap water.
5. Stop Solution **contains strong acid**. Wear eye, hand, and face protection.
6. For long term storage kit standards should be kept refrigerated, other components should be frozen.
7. Please perform centrifugation to collect liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for a good result. Use a mini-vortex at the lowest frequency.
10. Mix each sample and all components in the kits adequately and use a clean plastic container to prepare diluent.
11. Samples and standards should be assayed in duplicate, and the sequence of the regents should be added consistently.
12. Reuse of the dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.
14. The kit should be kept away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma 16. and other biological fluids in accordance with appropriate regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. Please prepare all kit components according to the specification. If the kits will be used several times, keep unused strips sealed and preserve with desiccants. Use within 2 months.

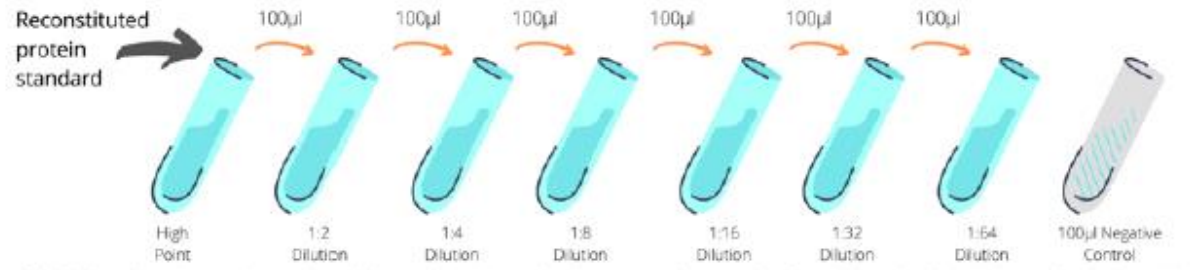
Experiment Materials

The following materials are required to carry out the aforementioned assay but are not included with this kit.

1. Microplate reader (measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000 µL.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

Reagent Preparation

1. Bring all reagents to room temperature before use. If crystals have formed in the concentrate bring the reagent to room temperature and mix gently until the crystals have completely dissolved. It is recommended to test in duplicates.
2. Standard working solution: Centrifuge the standard at 10,000xg for 1min. Add Standard/Sample Diluent 1.0mL into freeze-dried standard, sit for 10 minutes with gentle agitation prior to making dilutions (100 ng/mL). Prepare EP tubes containing Standard/Sample Diluent, and carry out a serial dilution according to the picture shown below (recommended concentration for standard curve: 100 ng/mL, 50 ng/mL, 25 ng/mL, 12.5 ng/mL, 6.25 ng/mL, 3.13 ng/mL, 1.56 ng/mL). Any remaining standard solution can be aliquoted and stored at -20°C to -70°C.



Two-fold dilution series: Each microvial starts with 100µl diluent. 100µl is carried over from the reconstituted protein standard, thoroughly mixed and then 100µl is transferred to the next. The process is repeated in each microvial in the sequence. No treatment is carried out on the negative control, which should hold 100µl of diluent.

Dilution Method

1. Concentrated Biotin-Conjugated Antibody (100x): Dilute 1:100 with the Biotin Conjugate Antibody Diluent before use, and the diluted solution should be used within 30 min.

No. of strips	Concentrated Biotin-Conjugate antibody (100x)	Biotin-Conjugate antibody diluent
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

2. Streptavidin-HRP Concentrated (100x): Dilute 1:100 with the Streptavidin- HRP Diluent before use, and the diluted solution should be used within 30 min

No. of strips	Concentrated Streptavidin-HRP (100x)	Streptavidin-HRP antibody diluent
2	20ul	1980ul
4	40ul	3960ul
6	60ul	5940ul
8	80ul	7920ul
10	100ul	9900ul
12	120ul	11880ul

3. Wash buffer: Dilute 1:24 with double distilled or deionized water before use.
4. Substrate Mixture Solution: Calculate the required amount before the experiment.
   Mix Substrate Reagent A and B with equal volumes prior to use. **Note: Do not open the vial until required.**

Wash Method

Aspirate each well and wash, repeating the process 2 times for a total of 3 washes. Wash by filling each well with Wash Buffer (350ul) using a squirt bottle, manifold dispenser, or autowasher. 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.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack and reseal.
2. Add 100 µL of each different concentration of standard working solution to the first 2 columns, so that each row has duplicates of equal concentration.
3. Add 100 µL of each of the samples to each of the wells to be used, in duplicate.
   Cover with the adhesive strip provided and incubate for 90 minutes at 37°C.
   (Note: solution should be added to the bottom of CLIA plate well, avoid touching the inside wall and foaming.) Remove all the liquid from each well. **Do not wash**.
4. Remove all the liquid from each well. Do not wash.
5. Add 100µL Biotinylated detection antibody to all the wells. Cover with new adhesive strip provided and incubate for 1 hour at 37°C.
6. Aspirate the solution from all of the wells.
7. Add 350 µL wash buffer to each well, and aspirate after 60-120 seconds, repeating the process 2 times for a total of 3 washes.
8. Add 100 µL HRP-conjugate working solution to each of the wells. Cover with a new adhesive strip and incubate for 30 minutes at 37°C.
9. We recommend that you ensure the Microplate reader is set up during this incubation stage.
10. Add 350 µL wash buffer to each well, and aspirate after 60-120 seconds. Repeat the process 4 more times, to total 5 washes.
11. Add 100 µL Substrate Mixture Solution to each well and incubate for no more than 5 minutes at 37°C. Protect from light.
12. Immediately determine the RLU value of each well.
13. Upon completion of the experiment ensure you return unused reagents to their appropriate storage locations.

Specificity

This assay has high sensitivity and excellent specificity for detection of 40LDL.
No significant cross-reactivity or interference between LDL and analogues was observed.

Typical Data

The standard curves are provided for demonstration only. A standard curve should be generated for each set of LDL assayed.

Concentration (ng/mL)	100	50	25	12.5	6.25	3.13	1.56	0
RLU 1	36038	16938	8289	3481	1932	1136	564	34
RLU 2	43322	18246	7659	4079	1896	1044	624	38
Average RLU	39680	17592	7974	3780	1914	1090	594	36
Corrected RLU	39644	17556	7938	3744	1878	1054	558	-

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, mid-range and highlevel mouse LDL were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, mid-range and highlevel mouse LDL were tested on 3 different plates, 20 replicates in each plate.

Inter-plate Precision

Inter-assay Precision: 3 samples with low, mid-range and high-level mouse LDL were tested on 3 different plates, 20 replicates in each plate.

Recovery

The recovery of mouse LDL spiked at three different levels in samples throughout the range of the assay was evaluated in various matrices.

Matrix	Recovery range (%)	Average (%)
Serum (n=5)	100-113	107
EDTA plasma (n=5)	97-113	104
Cell culture media (n=5)	96-107	101

Troubleshooting

Problem	Causes	Solutions
Poorly developed standard curve	Inaccurate pipetting.	Check pipetting volume consistency and accuracy.
	Improper standard dilution.	Gently mix the standard solution and dissolve the powder thoroughly in solution.
	Wells were not fully aspirated.	Completely aspirate wells in between stages.
Low fluorescence readings	Insufficient incubation time.	Ensure sufficient incubation time.
	Incorrect assay temperature.	Use recommended incubation temperature. Bring substrate to room temperature before use.
	Inadequate reagent volumes or inconsistent dilution.	Check pipettes and ensure correctly prepared.
Large CV	Inaccurate pipetting.	Check pipettes and technique.
High background	Concentration of target protein is too high.	Use recommended dilution factor.
	Plate is insufficiently washed.	Review the manual's washing process. If using a plate washer, check that the ports are not obstructed.
	Contaminated wash buffer.	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the kit.	All the reagents should be stored according to the instructions.
	Too long incubation time.	Ensure precise incubation time.