Quantikine® ELISA

Mouse LDL R Immunoassay

Catalog Number MLDLR0

For the quantitative determination of mouse Low Density Lipoprotein Receptor (LDL R) concentrations in cell culture supernates, tissue homogenates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

The low density lipoprotein receptor (LDL R) is the founding member of the LDL R family of widely expressed cell surface scavenger receptors (1-6). Although members of the family were originally identified as endocytic receptors, they also co-regulate adjacent cell-surface signaling molecules (3, 5). Many proteins in the LDL R family are cleaved by extracellular proteases to release soluble forms to the circulation, and many of these soluble forms are active (1, 7). A 140 kDa soluble form of LDL R that includes most or all of the extracellular domain (ECD) can be released from PMA-treated cell lines, such as the liver cell line HepG2 (7). This release is downregulated by inhibiting protein kinase C and/or metalloproteinases. Additionally, an antiviral 28 kDa soluble form of LDL R consisting of the ligand binding portion of the ECD is reportedly released from the cell surface in response to interferons (8, 9).

Mature LDL R is a 160 kDa type I transmembrane glycoprotein that contains cysteine-rich complement-like repeats (class A LDL domains), calcium-binding EGF repeats, and β-propeller structures (class B LDL repeats) in the ECD (4-6, 10). Class A domains bind apolipoproteins, while class B LDL repeats are likely involved in intracellular LDL release by interacting with class A domains at low pH (5, 8). A membrane-proximal Ser/Thr-rich region shows extensive O-linked glycosylation. In other family members, glycosylation of this region can inhibit ECD shedding (5, 6, 11). A cytoplasmic NPxY motif links the LDL R to clathrin pits for endocytosis, and binds to select adaptor proteins (1, 11, 12). The mouse LDL R ECD shares 78% and 87% amino acid identity with human and rat LDL R, respectively.

LDL R is constitutively and widely expressed. Its expression can be downregulated by LDL and upregulated by hormones such as insulin and estradiol, and by many cytokines (7, 13). Its ECD binds ApoB and ApoE, the proteins that predominate in lipid-carrying lipoproteins LDL and VLDL (low/very low density lipoprotein), respectively (1, 2, 5, 14). Hepatocyte LDL R is responsible for endocytosis and clearing of most plasma LDL cholesterol (2, 14). LDL R binds the ApoB portion of LDL and brings it into endocytic vesicles. At the low pH of the vesicle, it dissociates, allowing the LDL to be degraded while the LDL R is recycled back to the cell surface (5, 10, 13, 15). Lack of LDL R expression or function causes familial hypercholesterolemia (FH); over 1100 FH-related human LDL R mutations have been found (5, 13-16). Overexpression or hyperfunction of the protease PCSK9 (proprotein convertase subtilisin/kexin type 9) can also cause increased plasma cholesterol by lowering cell surface LDL R expression (16-18). PCSK9 alters the pH-sensitive LDL R conformation change in the endocytic vesicle, thus promoting LDL R degradation rather than recycling to the cell surface (17, 18).

The Quantikine® Mouse LDL R Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse LDL R levels in cell culture supernates, tissue homogenates, serum, and plasma. It contains NS0-expressed recombinant mouse LDL R and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse LDL R accurately. Results obtained using natural mouse LDL R showed dose-response curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse LDL R.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse LDL R has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any LDL R present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse LDL R is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of LDL R bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator 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.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse LDL R Microplate	894089	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse LDL R.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse LDL R Standard	894091	2 vials of recombinant mouse LDL R in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse LDL R Control	894092	2 vials of recombinant mouse LDL R in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse LDL R Conjugate	894090	12 mL of a polyclonal antibody specific for mouse LDL R conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-34	895265	11 mL of a buffered protein base with blue dye and preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 1000 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards and samples.

If using tissue homogenate samples, the following are also required:

- Cell Lysis Buffer 2 (R&D Systems®, Catalog # 895347).
- PBS

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - Prior to assay, tissues must be homogenized according to the directions in the Sample Values section.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Samples containing excessively high levels of protein are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

* See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse LDL R Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

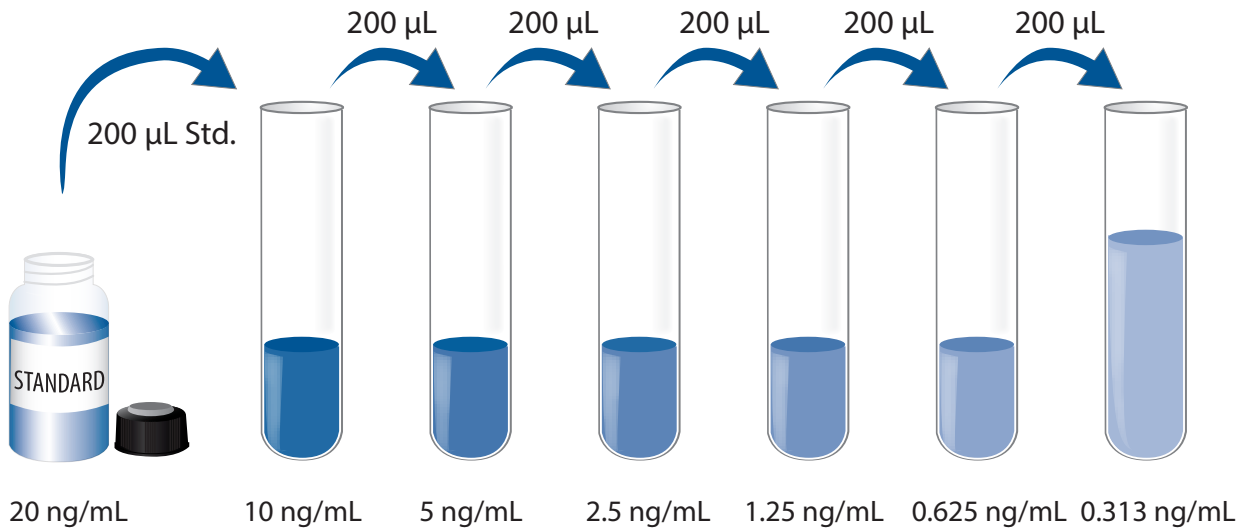
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Mouse LDL R Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse LDL R Standard with Calibrator Diluent RD5-26 (diluted 1:4). This reconstitution produces a stock solution of 20 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse LDL R Standard (20 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, control, 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, and reseal.
3. Add 50 µL of Assay Diluent RD1-34 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) 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.
6. Add 100 µL of Mouse LDL R Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature on the benchtop. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

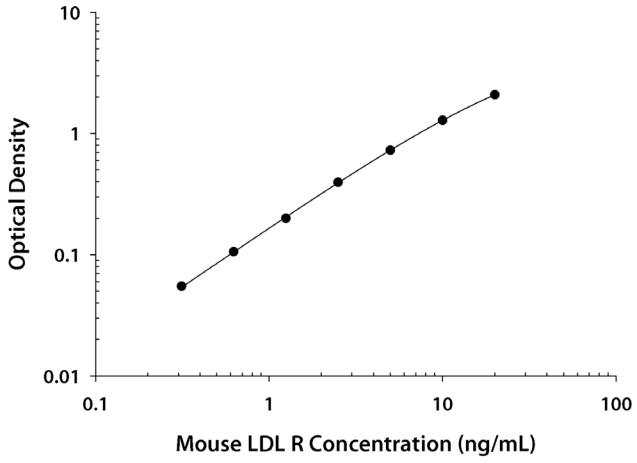
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse LDL R concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.046 0.047	0.047	—
0.313	0.100 0.103	0.102	0.055
0.625	0.148 0.157	0.153	0.106
1.25	0.238 0.255	0.247	0.200
2.5	0.428 0.458	0.443	0.396
5	0.755 0.794	0.775	0.728
10	1.301 1.362	1.332	1.285
20	2.091 2.179	2.135	2.088

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (ng/mL)	1.000	2.133	7.265	1.015	2.136	7.466
Standard deviation	0.053	0.097	0.355	0.083	0.148	0.428
CV (%)	5.3	4.5	4.9	8.2	6.9	5.7

RECOVERY

The recovery of mouse LDL R spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	104	80-114%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse LDL R were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Tissue homogenates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	97	99	98	97	99
	Range (%)	96-99	95-105	97-101	86-104	98-100
1:4	Average % of Expected	91	97	93	98	99
	Range (%)	89-96	89-101	89-96	92-106	96-102
1:8	Average % of Expected	89	92	95	99	102
	Range (%)	88-91	84-97	92-101	89-105	94-106
1:16	Average % of Expected	84	92	94	101	91
	Range (%)	80-87	85-96	83-101	90-116	80-102

SENSITIVITY

Eighty-one assays were evaluated and the minimum detectable dose (MDD) of mouse LDL R ranged from 0.008-0.064 ng/mL. The mean MDD was 0.026 ng/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-derived recombinant mouse LDL R produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse LDL R in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=23)	149	91.9-346	54.1
EDTA plasma (n=23)	118	71.2-250	44.9
Heparin plasma (n=23)	109	8.6-213	47.9

Cell Culture Supernates - Organs from mice were removed, cut into 1-2 mm pieces, and cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of mouse LDL R.

Tissue Type	(ng/mL)
Brain	5.66
Kidney	4.25
Liver	21.9
Lung	12.5
Spleen	1.31

Tissue Homogenates - Organs from mice were rinsed with PBS, cut into 1-2 mm pieces, and homogenized with a tissue homogenizer in PBS. An equal volume of Cell Lysis Buffer 2 was added and tissues were lysed at room temperature for 30 minutes with gentle agitation. Debris was then removed by centrifugation. Aliquots of the homogenates were removed and assayed for levels of mouse LDL R.

Tissue Type	(ng/mL)
Brain	148
Kidney	206
Liver	1464
Lung	380
Spleen	100

SPECIFICITY

This assay recognizes natural and recombinant mouse LDL R. This kit also recognizes PCSK9/LDL R Complex.

The factors listed below were prepared at 500 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant mouse LDL R control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

Apolipoprotein H
Proprotein Convertase 9/PCSK9
SR-AI/MSR1
VLDL R

Recombinant human:

Apolipoprotein B
Apolipoprotein B100
Apolipoprotein E
LDL R
PLA2G7

Natural proteins:

human LDL

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