This package insert follows the Pharmaceuticals, Medical devices and Other Therapeutic Products Act of Japan.

In Vitro Diagnostics
Marketing Approval No. 21900AMZ00070000

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This package insert must be read carefully prior to use.

MDA-LDL (oxidized LDL) kit
(Classification No.: 83018000)

Oxidized LDL ELISA "Daiichi"

General Precautions

1. This product is for in vitro diagnostic use, and must not be used for any other purposes.
2. Clinicians should make a comprehensive clinical decision based on assay results in conjunction with clinical symptoms and other examination results.
3. For the effects of an administered drug on the measured value, carefully read the Precautions for Use in the package insert of the drug, especially the section about the effects on laboratory test results.
4. This product should be used only as directed in this package insert. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.
5. If the reagent accidentally comes in contact with eyes and/or mouth, rinse immediately with ample water as first aid, and consult the doctor if required.
6. The calibrator, one of the components of this product, contains human-derived ingredients that have been confirmed to be negative for HBs antigens, HIV antibodies, and HCV antibodies. The calibrator should be considered potentially infectious and handled with great care in the same manner as the samples.
7. Perform a quality control test prior to assay to ensure accuracy.

Description (Kit Components)

Component	Ingredient
Antibody-Coated Plate	Wells coated with anti-MDA-LDL mouse monoclonal antibody
Enzyme-Labelled Antibody Solution	β-galactosidase-labelled anti-apolipoprotein B (apo B) mouse monoclonal antibody
Wash Buffer Concentrate	Phosphate Buffer Solution
Substrate Solution	o-Nitrophenyl-β-D-galactopyranoside
Stop solution	Sodium carbonate solution
Sample Dilution Solution	HEPES Buffer Solution
Sample Preservation Solution	Saccharose
Calibrator	Human serum

Intended Use

Measurement of MDA−LDL (oxidized LDL) in serum
(A diagnostic aid for predicting the risk of complications of coronary artery disease in patients with diabetes who have a history of coronary artery disease.)

Assay Principle

1. Assay Principle

Serum is processed with Sample Dilution Solution containing a surfactant to alter the structure of MDA−LDL. MDA-LDL in a sample binds to anti-MDA-LDL mouse monoclonal antibody (ML25) immobilized on the plate. Subsequently, β-galactosidase-labeled anti-apo B mouse monoclonal antibody (AB16) is added to form immune complexes consisting of the immobilized antibody, MDA-LDL, and enzyme-labelled antibody. Then O-nitrophenyl-β-D-galactopyranoside is added for development of color and the absorbance of the sample is measured to determine the MDA−LDL level.

2. Features

1) This product measures malondialdehyde-modified LDL (MDA-LDL), which is a representative form of oxidized LDL.
2) ML25 is an antibody that recognizes MDA-modified proteins, while AB16 recognizes MDA-LDL and LDL combined with apoB. An enzyme-linked immunosorbent assay (ELISA) combining both antibodies is used to specifically measure MDA-LDL.

Procedural Precautions *

1. Properties of Samples and Sampling Methods

1) Samples
(1) Always use serum. Do not use plasma.
(2) Serum should be isolated after the blood is completely clotted. Serum should be isolated within 6 hours after blood collection.
2) Storage of samples
(1) Fresh samples
Measure the sample within 8 hours after blood collection if it is stored at 15–25°C and within 3 days if it is stored at 2–8°C.
(2) Stored samples
In order to store a sample, either of the following procedures should be performed within 8 hours after blood collection.
(1) Store the sample after adding Sample Preservation Solution. Dilute the sample with Sample Preservation Solution at a 3:1 ratio (e.g., 300 μL of the sample and 100 μL of Sample Preservation Solution), and then freeze the diluted sample for storage. Measure the sample within 5 months if it is stored at –80°C and within 1 month if

it is stored at –20°C.

(2) Freeze the undiluted sample at –20°C or below for storage. Avoid thawing/refreezing during storage. When the sample is stored at –20°C, the following procedures should be performed within 1 week. After thawing, dilute the sample with Sample Preservation Solution at a 3:1 ratio (e.g., 300 µL of the sample and 100 µL of Sample Preservation Solution) before measurement. When the sample is refrozen for storage, perform measurement within 5 months if it is stored at –80°C and within 1 month if it is stored at –20°C.

(3) Diluted samples should be brought to room temperature (15–30°C) before use.

2. Interfering substances

Assay results are not affected by free bilirubin (up to 20 mg/dL), conjugated bilirubin (up to 20 mg/dL), hemoglobin (up to 500 mg/dL), or formazin turbidity (up to 3000 FTU).

3. Others

1) Prepare a new calibration curve for every assay.
2) When measuring multiple samples, be sure to maintain the specified constant reaction time for each well.
3) Completely remove the Wash Buffer Solution after the washing step.
4) Do not perform the assay under direct sunlight.
5) Place the sample, the diluted calibrator solution, and each reagent in the center of each well while avoiding contact with the walls.
6) If the MDA-LDL concentration in the sample exceeds the measurement range, dilute the sample with a Sample Dilution Solution and perform re-measurement.

Dosage/Administration (Assay Procedure) *

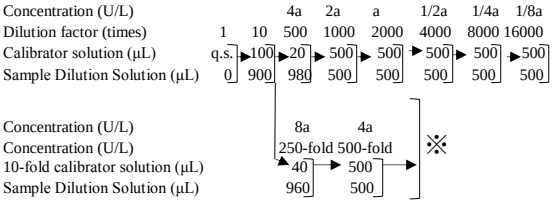
1. Instruments and reagents required for measurement

1) Variable micropipettes (20, 200, and 1000 µL)
2) Test tube
3) Measuring cylinder (1000 mL)
4) Plate seal or plate cover
5) Microplate reader (dominant wavelength of 415 nm and complementary wavelength of 600 nm, or single wavelength of 415 nm)
6) Purified water

2. Preparation of reagents

1) Antibody-Coated Plate: Ready to use Unused wells are stable at 2–10°C for 2 weeks when stored in an air-tight container.
2) Enzyme-Labelled Antibody Solution: Ready to use
3) Wash Buffer: Add 400 mL of purified water to 100 mL of Wash Buffer Concentrate before use.
4) Substrate Solution: Ready to use
5) Stop Solution: Ready to use
6) Sample Dilution Solution: Ready to use
7) Sample Preservation Solution: Ready to use
8) Calibrator Solution: Add 0.5 mL of purified water to the calibrator to make the calibrator solution. Dilute the solution with the dilution solution according to the following example and let it stand for 1 hour at room temperature (15–30°C) before use.

Example:
(Indicated value for calibrator a U/L)



As a reagent blank, use unmodified Sample Dilution Solution together with diluted calibrator solution prepared according to the above example.

※When the indicated value for the calibrator is less than 83 U/L, prepare a calibration curve starting from a 250-fold dilution that includes the upper limit of measurement (330 U/L) in the calibration range.

3. Assay Procedure

Preparation of reagents

1) Fresh samples

To make a sample, take serum and dilute it with Sample Dilution Solution to a final dilution ratio of 1:2000 according to the following steps. Let it stand for 1 hour at room temperature (15–30°C) before use.

(1) Dilute the sample 50-fold by adding 980 µL of Sample Dilution Solution to 20 µL of the sample.

(2) Dilute the 50-fold diluted sample prepared in Step (1) by a further 40-fold by adding 780 µL of Sample Dilution Solution to 20 µL of the 50-fold diluted sample.

(3) Let it stand for 1 hour at room temperature (15–30°C) and use as the sample.

	(50-fold dilution)	(40-fold dilution)	(Final dilution ratio 1:2000)
Sample dilution Solution	980 µL	780 µL	Let it stand for 1 hour at room temperature (15–30°C) and use as the sample.
Sample	20 µL	20 µL	

2) Stored samples

Before use, stored samples to which Sample Preservation Solution was added should be diluted 1500-fold with Sample Dilution Solution to achieve a final dilution ratio of 1:2000 according to the following steps. Let it stand for 1 hour at room temperature (15–30°C) before use.

(1) Dilute the stored sample 50-fold by adding 980 µL of Sample Dilution Solution to 20 µL of the stored sample.

(2) Dilute the 50-fold diluted stored sample prepared in Step (1) by a further 30-fold by adding 580 µL of Sample Dilution Solution to 20 µL of the 50-fold diluted

stored sample.

(3) Let it stand for 1 hour at room temperature (15–30°C) and use as the sample.

	(50-fold dilution)	(30-fold dilution)	(Final dilution ratio 1:2000)
Sample Dilution Solution	980 µL	580 µL	Let it stand for 1 hour at room temperature (15–30°C) and use as the sample.
Sample	20 µL	20 µL	

Measurement

(1) All reagents should be brought to room temperature (15–30°C) before use.

(2) Dispense 300 µL of the Wash Buffer into each well of the antibody-coated plate and then discard the buffer. Repeat this step three times to wash the wells. Remove the Wash Buffer completely, and then follow the steps below.

(3) Dispense 100 µL of the sample or diluted calibrator solution into each well and then let the plate stand for 2 hours at room temperature (15–30°C). Make sure that the difference in reaction time among the wells does not exceed 10 minutes. Duplicate assays are required for diluted calibrator solution.

(4) Remove the solution from the wells, and dispense 300 µL of Wash Buffer into each well and wash three times as in Step (2).

(5) Remove the Wash Buffer, dispense 100 µL of Enzyme-Labeled Antibody Solution into each well, and then let the plate stand for 1 hour at room temperature (15–30°C).

(6) Remove the solution from the wells, and dispense 300 µL of Wash Buffer into each well and wash three times as in Step (2).

(7) Remove the Wash Buffer, dispense 100 µL of Substrate Solution into each well, and then let the plate stand for 2 hours at room temperature (15–30°C).

(8) Dispense 100 µL of Stop Solution to terminate the reaction and maintain a constant response time among the wells.

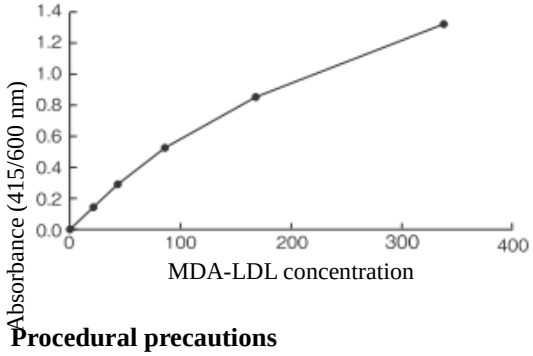
(9) Read the absorbance at a dominant wavelength of 415 nm and a complementary wavelength of 600 nm, or only at the dominant wavelength of 415 nm, using a microplate reader with a reagent blank as the control. Sample Dilution Solution is used as the reagent blank.

4. Method of calculating the MDA-LDL concentration

1) Create a graph with the concentration of MDA−LDL on the X-axis and the absorbance on the Y-axis. Then plot the parameters for the diluted calibrator solutions and fit a smooth curve to the data points to obtain the calibration curve.

2) Read the concentration corresponding to the measured absorbance of the sample on the calibration curve to obtain the concentration of MDA-LDL.

Example of a calibration curve



5. Procedural precautions

1) All reagents should be brought to room temperature (15–30°C) before use. If insoluble matter has formed in a refrigerated Sample Dilution Solution, ensure complete dissolution before use.

(2) Add exactly 0.5 mL of purified water to 1 vial of the calibrator, fit the inner stopper, mix gently, and let stand for 10 minutes at room temperature (15–30°C). After confirming that the contents of the vial have been completely dissolved, gently mix the solution by inversion before use. At this time, avoid vigorous mixing.

3) Dilute the sample with the Sample Dilution Solution through gentle mixing by inversion. Do not mix vigorously with a vortex mixer.

4) When the antibody-coated plate is let stand for measurement, cover it with a plate seal or plate cover to avoid evaporation of the Reactant Solution.

Assessment of Assay Results *

1. Reference standard range[10)]

Men aged < 45 years or women aged <55 years: 64 ± 18 U/L (mean ± SD) (N = 134)

Men aged ≥ 45 years or women aged ≥ 55 years: 83 ± 22 U/L (mean ± SD) (N = 122)

2. Precautions for Assessment

There may be reactions or interfering reactions with non-target substances. If assay results appear to be unreliable, repeat the measurement (if necessary, after dilution) or try another analytical methods.

Clinical Significance[10)] *

Oxidized LDL is a general term for LDL that has undergone oxidative degradation. Lipid peroxidation products modify apo B, the main protein in LDL, resulting in oxidized LDL. Malondialdehyde (MDA) has been identified as a representative lipid peroxidation product, and LDL in which apo B has been modified by MDA is called malondialdehyde-modified LDL (MDA−LDL). MDA-LDL is considered to be a typical form of oxidized LDL.

In patients with diabetes mellitus undergoing percutaneous coronary intervention (PCI), the relationship between the pre-procedural MDA-LDL level and restenosis after PCI was investigated. MDA-LDL levels were higher in patients with restenosis than in those without restenosis and patients with an MDA-LDL level ≥ 110 U/L had a higher risk of restenosis than those with an

MDA-LDL level < 110 U/L (relative risk = 5.3).
In patients with diabetes and a history of coronary artery disease (CAD), the relationship between the MDA-LDL level and cardiac events was investigated during a 4-year follow-up period, revealing a significantly higher frequency of cardiac events in patients with an MDA-LDL level ≥ 110 U/L than in those with an MDA-LDL level < 110 U/L.
These findings demonstrated that MDA-LDL is a useful prognostic marker for cardiac events in patients with diabetes and a history of CAD.

Performance[10] *

1. Sensitivity
1) When the test is performed using Sample Dilution Solution, the absorbance is ≤ 0.15.
2) When the test is performed using a sample with a known concentration, the absorbance is 0.4–1.8 per 200 U/L of MDA-LDL.

2. Accuracy: 80–120 % of the expected assay value

3. Within-run Reproducibility:
Coefficient of variation ≤ 15 %

(Test methods used for 1.–3. are in-house methods.)

4. Measurement Range[10]:
10–330 U /L

5. Standard Material
MDA−LDL (in-house standard material)

Precautions for Use or Handling *

1. Precautions for Handling (to Ensure Safety)
1) All samples used in the test should be handled as a material possibly infected with HIV, HBV, HCV, or other viruses. To prevent infection, use disposable gloves and avoid mouth pipetting during the test.
2) Proclin 300, which possesses skin-irritative potential, is added as an antiseptic agent in the Enzyme-Labelled Anti-body Solution, Wash Buffer Concentrate, Substrate Solution, Sample Dilution Solution, Sample Preservation Solution, and Calibrator. Therefore, if the reagent comes in contact with skin or clothes, rinse immediately with ample water, and consult the doctor if skin irritation develops.

2. Precautions for use
1) This product should be stored as directed, without freezing. Freezing can deteriorate the reagents, which can produce inaccurate results. Therefore, avoid using the reagents which have been previously frozen.
2) Do not use expired reagents. Use of such reagents cannot guarantee the reliability of measurement values.
3) Do not replenish the reagents.
4) Do not mix materials from different kit lot numbers.
5) Do not exchange caps between reagents.
6) Do not allow the cap of the reagent bottle to come into contact with other reagents or samples.
7) Do not perform the assay under direct sunlight

3. Precautions for Disposal
1) Before disposal, used samples and their containers must be immersed in sodium hypochlorite solution at a concentration of greater than 0.1% for longer than 1 hour or autoclaved at 121ºC for 20 minutes.
2) To prevent infections from spilled samples or solutions containing samples, wipe the spilled area thoroughly with disinfectants such as sodium hypochlorite solution at a concentration of greater than 0.1%.
3) The reagents and treated samples should be discarded as medical waste or industrial waste according to the waste disposal regulations.
4) The reagents should be disposed of in accordance with the Water Pollution Control act or related regulations.

4. Other precautions
Do not use the containers for other purposes.

Storage and Shelf Life *

1. Storage temperature: 2–10°C
2. Shelf life: 2 years from the date of manufacture
(The expiration date is printed on the outer package.)

Packaging

For 96 assays

Name		Package
Oxidized LDL ELISA "Daiichi"	Antibody-Coated Plate	1 × 96 wells
	Enzyme-Labelled Antibody Solution	1 × 11 mL
	Wash Buffer Concentrate	For 2 × 500 mL
	Substrate Solution	1 × 11 mL
	Stop solution	1 × 11 mL
	Sample Dilution Solution	2 × 100 mL
	Sample Preservation Solution	1 × 50 mL
	Calibrator	For 1 × 0.5 mL

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