KAMIYA BIOMEDICAL COMPANY

# Human Very Low Density Lipoproteins (VLDL) ELISA

**For the quantitative determination of human VLDL in biological samples**

Cat. No. KT-50962

**For Research Use Only. Not for use in diagnostic procedures.**

# Product Information

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## INTENDED USE

This ELISA kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of human VLDL in biological samples. For research use only. Not for use in diagnostic procedures.

## PRINCIPLE

The assay sample and buffer are incubated together in the anti-VLDL antibody coated plate for sixty minutes and washed. The diluted C-Peptide-HRP conjugate is then added to each well and incubated. After the incubation period, the wells are decanted and washed three times. The wells are then incubated with a substrate for the enzyme. The product of the enzyme-substrate reaction is a blue colored complex. Finally, a stopping solution is added to stop the reaction. The stop solution changes the color from blue to yellow. The intensity of the color is then measured spectrophotometrically at 450 nm in a microplate reader. The intensity of the color is inversely proportional to the VLDL concentration since VLDL from calibrators and samples compete with the VLDL-HRP conjugate for the anti-VLDL antibody binding site. Since the number of sites is limited, as more sites are occupied by VLDL from the samples or calibrators, fewer sites are available to bind the C-Peptide-HRP conjugate. Calibrators of known VLDL concentrations are run concurrently with the samples being assayed and a calibration curve is plotted relating the intensity of the color (OD) to the concentration of C-Peptide. The unknown VLDL concentration in each sample is interpolated from this curve.

## COMPONENTS

| Reagents | Quantity |
|---|---|
| Pre-coated 96-well plate | 1 |
| Calibrator 1 (0 ng/mL) | 1 |
| Calibrator 2 (50 ng/mL) | 1 |
| Calibrator 3 (100 ng/mL) | 1 |
| Calibrator 4 (200 ng/mL) | 1 |
| Calibrator 5 (250 ng/mL) | 1 |
| Calibrator 6 (500 ng/mL) | 1 |
| Enzyme Conjugate | 1 x 6 mL |
| Substrate A | 1 x 6 mL |
| Substrate B | 1 x 6 mL |
| Stop Solution | 1 x 6 mL |
| Wash Buffer (100X concentrate) | 1 x 10 mL |
| Lysis Buffer | 1 x 10 mL |

## STORAGE

All reagents should be stored at 4°C upon receipt. For expiration date refer to kit label.

# SAMPLE COLLECTION AND STORAGE

## Serum
Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1,000 x g. Remove serum and assay immediately or store samples in aliquots at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

## Plasma
Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000 x g at 4℃ within 30 minutes of collection. Assay immediately or store samples in aliquots at -20℃ or -80℃ for later use. Avoid repeated freeze/thaw cycles.

## Tissue Homogenates
Use between 0.3 and 0.5 g of tissue. Evenly cut the tissue into small pieces. Add 500 µL of PBS (1:10-1:20) or saline to wash the tissue. Wash 1-2 times as necessary. Centrifuge at 5,000 RPM for 10 minutes and remove the supernate. Assay immediately or aliquot and store samples at -20℃.

## Cell Culture Supernates and Other Biological Fluids
Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20℃ or -80℃. Avoid repeated freeze-thaw cycles.

## ⚠ Note:
1. Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination.
2. When performing the assay, slowly bring samples to room temperature.
3. Sample hemolysis will influence the result, so hemolytic specimen cannot be detected.
4. Do not use heat treated specimens.

# MATERIALS REQUIRED BUT NOT SUPPLIED
1. Microplate reader with 450 nm filter.
2. Precision pipettes to deliver 1-2 mL volumes.
3. Adjustable 10-100 mL pipettes for reagent preparation.
4. 100 mL and 1 L graduated cylinders.
5. Calibrated adjustable precision pipettes with disposable tips (multi-channel is desirable for large assays).
6. 37℃ incubator.
7. Absorbent paper.
8. Distilled or deionized water
9. Data analysis tools such as graphing software, or graph paper (linear, log-log, semi-log, or log-logit as desired).
10. Tubes to prepare Calibrators or sample dilutions.

# Precautions
1. Do not substitute reagents from one kit lot to another. Calibrators, conjugate, and microtiter plates are matched for optimal performance. Use only reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 4°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer

complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.

9. All samples should be disposed of in a manner that will inactivate viruses.
10. Solid waste: Autoclave 60 min at 121°C.
11. Liquid waste: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate the viruses before disposal.
12. Substrate solution is easily contaminated. If bluish prior to use, do not use.
13. Substrate B contains 20% acetone, keep this reagent away from sources of heat or flame.
14. Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C).

## ASSAY PROCEDURE

1. Prepare all calibrators before starting assay procedure (see Reagent Preparation). It is recommended that all calibrators and samples be added in duplicate to the microtiter plate.
2. First, secure the desired number of coated wells in the holder, then add 100 µL of calibrators and samples to the appropriate well of the antibody coated microtiter plate.
3. Add 50 µL of conjugate to each well. Mix well. Complete mixing in this step is important. Cover and incubate for 1 hour at 37°C.
4. Prepare substrate solution no more than 15 minutes before end of incubation (see Reagent Preparation).
5. Wash the microtiter plate using one of the specified methods indicated below:
6. Manual Washing: Remove incubation mixture by aspiration contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with distilled or de-ionized water, then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a total of five washes. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. Note: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
7. Automated Washing: Aspirate all wells, then wash plate five times using distilled or de-ionized water. Always adjust your washer to aspirate as much liquid as possible and set fill volume to 350 µL/well/wash (range: 350-400 µL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper and paper towels until no moisture appears. It is recommended that the washer be set for a soaking time of 15-30 seconds or shaking time of 5 seconds between washes.
8. Add 50 µL substrate A and B to each well. Cover and incubate for 15 minutes at 20-25°C.
9. Add 50 µL stop solution to each well. Mix well.
10. Read the optical density (OD) at 450 nm using a microtiter plate reader within 30 minutes.

## CALCULATION OF RESULTS

1. Calculate the mean absorbance value A450 for each set of calibrators and samples.
2. Divide the average A450 value for each calibrator, control and sample by the average A450 of calibrator 0 and multiply by 100 to obtain %B/B0 for each sample.
3. Prepare a calibration curve by plotting the average absorbance of each calibrator versus the corresponding concentrations of the calibrators on linear-log graph paper or the %B/B0 value for each calibrator versus the corresponding concentration of the calibrator on linear-log or logit-log paper. logit= ln(B/B0)/(1-B/B0).
4. Any values obtained for diluted samples must be further converted by applying the appropriate dilution factor in the calculations.
5. The calibrator density is on the x-axis, while the B/B0 is on the y-axis
6. The sensitivity of this assay is 0.01 ng/mL

## REAGENT PREPARATION

All reagents must be allowed to reach room temperature before use. Additional information for individual reagents can be found on vial labels.

### ⚠ Note:

1. This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drain with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin, and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

2. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

## IMPORTANT NOTES

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. Microtiter plates should be allowed to come to room temperature before opening the foil bags. Once the desired number of strips has been removed, immediately reseal the bag and store at 4°C to maintain plate integrity.
3. Samples should be collected in pyrogen/endotoxin free tubes.
4. Samples should be frozen if not utilized shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to use.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to use.
6. It is recommended that all calibrators, controls, and samples be run in duplicate.
7. When pipetting reagents maintain a consistent order from well-to-well. This ensures equal incubation time for all wells.
8. Cover or cap all reagents when not in use.
9. Do not mix or interchange different reagent lots from various kit lots.
10. Do not use reagents after the kit expiration date.
11. Read absorbances within 30 minutes of assay completion.
12. The provided protocols should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
13. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. Never insert absorbent paper directly into wells.
14. Because stabilized Chromogen is light sensitive, avoid prolonged exposure to light. Also avoid contact between stabilized Chromogen and metal, or color may develop.
15. Incomplete washing will adversely affect the test outcome. All washing must be performed with wash buffer provided.
16. Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering VLDL aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.
17. After aspiration, fill the wells with at least 0.35 mL of diluted wash solution. Let soak for 15-30 seconds, the aspirate the liquid. Repeat as directed under Assay Procedure. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
18. Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.
19. If using an automated washer, the operating instructions for washing equipment should be carefully followed.
20. Assay Procedure Preliminary notes: Do not mix reagents from different lots. It is recommended that assays be performed in duplicate. Calibrators and samples must be assayed at the same time. Avoid exposing the substrate to direct sunlight.

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