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ab242310 Oxidized HDL Assay Kit (HNE-HDL, Human)

View Oxidized HDL Assay Kit (HNE-HDL, Human) datasheet:
www.abcam.com/ab242310

For the measurement of HNE-HDL in human plasma, serum or other biological fluid samples.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Oxidized HDL Assay Kit (HNE-HDL, Human) (ab242310) is an enzyme immunoassay developed for the detection and quantitation of human OxHDL in plasma, serum or other biological fluid samples.

The kit contains an HNE-HDL standard and has a detection sensitivity limit of 2 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

2. Protocol Summary

Prepare all reagents, samples and standards as instructed.



Add 100 µL of HNE-HDL standard or sample to the anti-HNE-HDL Antibody coated plate. Cover and incubate for 2 hours at room temperature.



Washing steps with 1X Wash Buffer. Then add 100 µL Blocking Reagent to each well. Incubate for 1 hour at room temperature.



Washing steps with 1X Wash Buffer. Add 100 µL Biotinylated Anti-Human Apo AI antibody to each well. Incubate for 1 hour at room temperature.



Washing steps with 1X Wash Buffer. Add 100 µL Streptavidin-Enzyme Conjugate to each well. Incubate for 1 hour at room temperature.



Washing steps with 1X Wash Buffer. Add 100 µL room temperature Substrate Solution to each well. Incubate for 5-20 minutes at room temperature.



Stop the enzyme reaction by adding 100 µL of Stop Solution to each well. Read absorbance immediately on a microplate reader at 450 nm.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at +4°C (apart from Blocking Reagent and HNE-HDL Standard which should be stored at -20°C) immediately upon receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.
- Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage condition
Anti-HNE Antibody Coated Plate	96 well	+4°C
Biotinylated Anti-Human ApoAI Antibody (1000X)	20 µL	+4°C
Streptavidin-Enzyme Conjugate	20 µL	+4°C
Assay Diluent	50 mL	+4°C
10X Wash Buffer	100 mL	+4°C
Substrate Solution	12 mL	+4°C
Stop Solution	12 mL	+4°C
Precipitaion Solution 1	250 µL	+4°C
Precipitaion Solution 2	2.5 mL	+4°C
Precipitaion Solution 3	650 µL	+4°C
Buffer Solution	5 mL	+4°C
5X Wash Solution	1.2 mL	+4°C
Blocking Reagent (100X)	200 µL	-20°C
HNE-HDL Standard	20 µL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microcentrifuge
- Microplate reader capable of reading O.D. at 450 nm (620 nm as optional reference wave length)

6. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.
- Any components not listed here are ready to use as supplied.

6.1 1X Wash Buffer:

6.1.1 Dilute the 10X Wash Buffer Concentrate to 1X with deionized water. Stir to homogeneity.

6.2 Blocking Reagent:

6.2.1 Immediately before use dilute the Blocking Reagent 1:100 with PBS.

6.3 Biotinylated Anti-Human ApoAI Antibody:

6.3.1 Immediately before use dilute the Biotinylated Anti-Human ApoAI Antibody 1:1000 with Assay Diluent.

6.4 Streptavidin-Enzyme Conjugate:

6.4.1 Immediately before use dilute the Strepatavidin-Enzyme Conjugate 1:1000 with Assay Diluent.

6.5 Resuspension Buffer:

6.5.1 Dilute Precipitation Solution 3 1:100 and Precipitation Solution 2 1:10 in Buffer Solution. For example, add 1 µL of Precipitation Solution 3 and 10 µL of Precipitation Solution 2 to 89 µL of Buffer Solution. Mix well. Prepare only enough for immediate use and do not store unused buffer.

6.6 1X Wash Solution:

6.6.1 Dilute the 5X Wash Solution to 1X with deionized water. Mix well. Store unused solution at 4°C.

Δ Note: Do not store diluted solutions unless stated otherwise.

7. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

7.1 Preparation of the DCF Standard Curve

7.1.1 Dilute the 0.5 mg/mL HNE-HDL Standard 1:100 to 5 µg/mL in Assay Diluent. For example, add 4 µL of 0.5 mg/mL HNE-HDL Standard to 396 µL of Assay Diluent. Transfer 200 µL of each DCF standard to a 96-well plate suitable for fluorescence measurement.

7.1.2 Prepare a dilution series of HNE-HDL Standards in the concentration range of 0 to 80 ng/mL in Assay Diluent as shown in the table below.

Standard #	0.5 µg/mL HNE-HDL Standard (µL)	Assay Diluent (µL)	HNE-HDL Standard (ng/mL)
1	16	984	80
2	250 of standard #1	250	40
3	250 of standard #2	250	20
4	250 of standard #3	250	10
5	250 of standard #4	250	5
6	250 of standard #5	250	2.5
7	250 of standard #6	250	1.25
8	0	250	0

8. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples for the most reproducible assay.

8.1 Plasma or serum:

8.1.1.1 For preparation of plasma, collect blood with heparin or EDTA, centrifuge for 10 minutes at 1000 x g at 4°C, and isolate plasma.

8.1.1.2 For serum, harvest serum and centrifuge for 10 minutes at 1000 x g at 4°C.

8.1.1.3 Transfer 200 µL of plasma or serum to an eppendorf tube, add 5 µL of Precipitation Solution 1, and add 10 µL of Precipitation Solution 2, mixing well.

8.1.1.4 Incubate at room temperature for 5 minutes (precipitation will occur). Centrifuge for 10 minutes at 6000 x g at 4°C (pellet should be visible).

8.1.1.5 Carefully collect the supernatant and transfer to a new eppendorf tube. Add 12 µL of Precipitation Solution 3 and 30 µL of Precipitation Solution 2, mixing well. Incubate at room temperature for 2 hours.

8.1.1.6 Centrifuge for 30 minutes at 18-20000 x g at 4°C (pellet should be visible). Discard the supernatant and resuspend pellet in 100 µL resuspension buffer. Mix thoroughly by pipetting up and down.

8.1.1.7 Centrifuge for 10 minutes at 6000 x g at 4°C. Discard supernatant and resuspend pellet in 120 µL of 1X Wash solution. Shake tube for 30 minutes at 4°C. Shaking speed should be sufficient to dissolve pellet, but not so vigorous that bubbles form.

8.1.1.8 Centrifuge tube again for 10 minutes at 6000 x g at 4°C. Transfer the supernatant to a new tube and store at 4°C if running the ELISA on the same day; otherwise store at -80C for up to 2 months.

8.1.1.9 Further dilute the sample 1:50 to 1:200 in Assay Diluent before running the ELISA. Assay immediately and do not store solutions.

Δ Note: The above are only guidelines and may be altered to optimize or complement the user's experimental design.

9. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

9.1 Please refer to the Sample Preparation section. Crude samples require the isolation steps described above prior to running the assay.

9.2 Add 100 µL of HNE-HDL standard or unknown sample to the Anti-MDA Antibody Coated Plate. Each HNE-HDL standard, blank and purified unknown sample should be assayed in duplicate.

9.3 Cover with a plate cover and incubate at room temperature for 2 hours on an orbital shaker.

9.4 Wash microwell strips 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

9.5 Add 100 µL of diluted Blocking Reagent to each well. Cover with a plate cover and incubate at room temperature for 1 hour on an orbital shaker.

9.6 Wash microwell strips 5 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.

9.7 Add 100 µL of the diluted Biotinylated Anti-Human Apo AI antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.

9.8 Wash the strip wells 5 times according to step 9.6 above.

9.9 Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.

9.10 Wash the strip wells 5 times according to step 9.6 above. Proceed immediately to the next step.

9.11 Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature on an orbital shaker. Actual incubation time may vary from 5-20 minutes.

Δ Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.

9.12 Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).

9.13 Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

10. Data Analysis

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.

10.1 Average the duplicate reading for each standard, and sample.

10.2 Subtract the mean value of the blank (Standard #8) from all standards, controls and sample readings. This is the corrected absorbance.

10.3 Plot the corrected values for each standard as a function of the final concentration of HNE-HDL.

10.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

10.5 Apply the corrected sample OD reading to the standard curve to get HNE-HDL amount in the sample wells.

10.6 Concentration of HNE-HDL in ng / mL in the test samples is calculated as:

$$HNE - HDL\ concentration\ (A) = \frac{B}{V} * D$$

Where:

A = HNE-HDL concentration in ng/mL.

B = amount of HNE-HDL in the sample well calculated from standard curve in ng/mL

V = sample volume added in the sample wells in µL.

D = sample dilution factor if sample is diluted to fit within the standard curve range (prior to reaction well set up).

11. Typical Data

Data provided **for demonstration purposes only.**

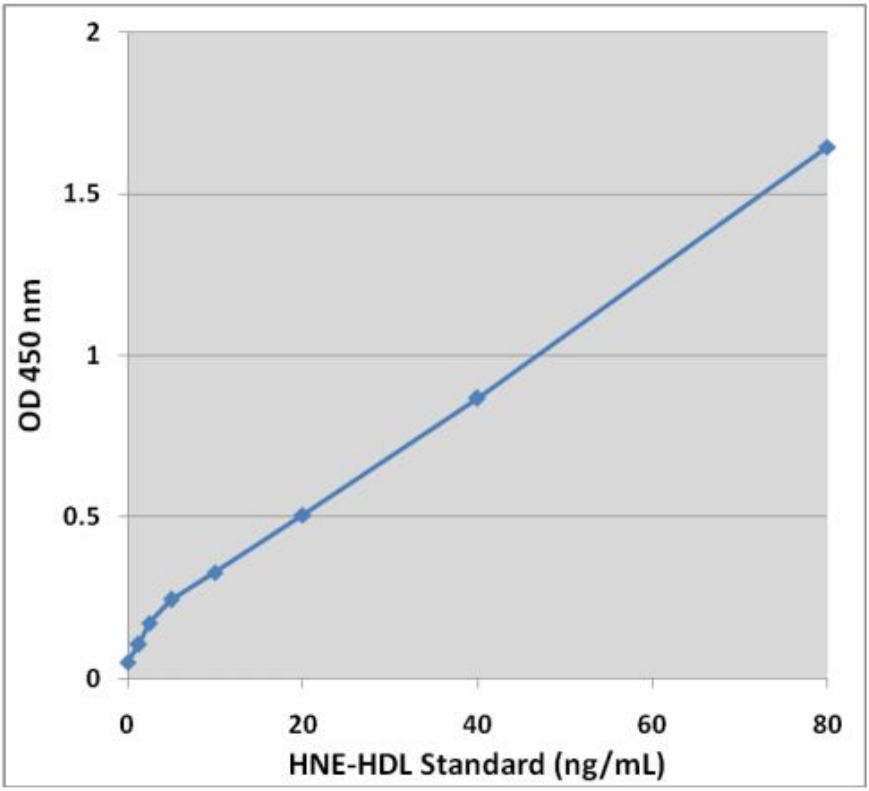

Figure 1. Human HNE-HDL Standard Curve.

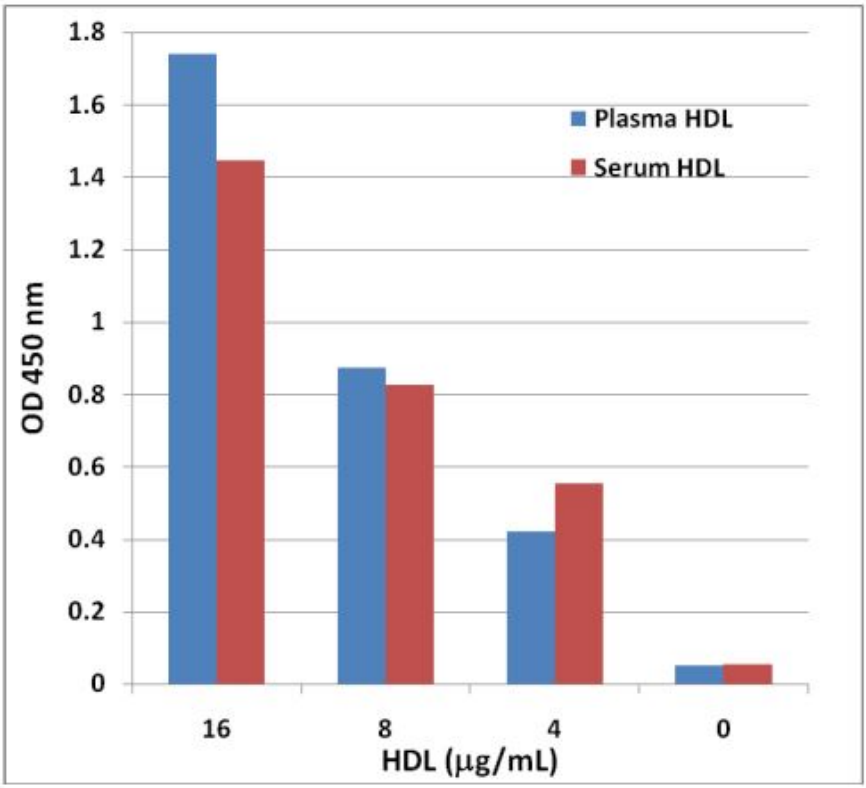

Figure 2. HDL from human serum and plasma samples was isolated according to the Sample Preparation Section. Samples were diluted from 5 mg/mL to 16 µg/mL in Assay Diluent, and then two-fold serial dilutions were prepared.

12. Notes

Technical Support

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