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Working

UC Davis - LDL Protocol

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ABSTRACT

Summary:

LDL and VLDL are separated from HDL using a precipitation reagent. Then the HDL fraction is measured for either TC or TG using the same reagents for total cholesterol or triglyceride.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=94&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Calibrator	TR43002	Fisher Diagnostics
TC Reagents	TR13421	Fisher Diagnostics
TG Reagents	TR22421	Fisher Diagnostics
2X LDL/VLDL Precipitation Buffer	ab105138	Abcam
PBS		
Microplate		
Platereader		

MATERIALS TEXT

Note:

Fisher Scientific, RRID:SCR_008452
Abcam, RRID:SCR_012931

1 Add 25µl 2X precipitation buffer to 25µl of sample using a positive displacement pipet.

2 Vortex and let sit at RT for 10 minutes.

3 Centrifuge at 2000×g for 10 minutes at 4°C.

4 Pipet supernatant into new tube, this is the HDL fraction.

5 Add 5 µl of calibrator and sample to each well.

IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

6 Add 300 µl of TC or TG reagent to each well. Incubate at 37°C for 5 minutes. Read at 540 nm.

IMPORTANT: If samples are hemolyzed, pipet a blank well with 5µl sample and 300µl PBS

7 Subtract blank readings from final readings. The assay will be linear so the unknown samples can be calculated as (Sample Absorbance ÷ Calibrator Absorbance) × Calibrator Concentration.

8 HDL samples are diluted ½ so multiply these by 2 to get the final value. Subtract this from the total triglyceride or cholesterol value to get the LDL/VLDL value.