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UC Davis - HDL Protocol

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Protocol status: Working
We use this protocol and it's working

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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.

Materials

MATERIALS

- Calibrator **Fisher Diagnostics Catalog #**TR43002
- TC Reagents **Fisher Diagnostics Catalog #**TR13421
- TG Reagents **Fisher Diagnostics Catalog #**TR22421
- 2X LDL/VLDL Precipitation Buffer **Abcam Catalog #**ab105138
- PBS
- Microplate
- Platereader

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.