D3406 HDL-C and LDL-C/VLDL-C

And

D3407 to "HDL-TG and LDL-TG/VLDL-TG Protocols

Version: 1
Replaced by version N/A
Edited by: Peter Havel - UC Davis Metabolism and Endocrinology Core

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.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
Calibrator	Fisher Diagnostics	TR43002
TC Reagents	Fisher Diagnostics	TR13421
TG Reagents	Fisher Diagnostics	TR22421
2X LDL/VLDL Precipitation Buffer	AbCam	ab105138
PBS		
Microplate		
Platereader		

Protocol:

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.

Reagent Preparation:

Reagent – ready to use
PBS – ready to use