UC Davis - HDL Protocol

Peter Havel

1University of California, Davis

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

- 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 Contributed by users
- Microplate Contributed by users
- Platereader Contributed by users

Note:

Fisher Scientific, RRID:SCR_008452
Abcam, RRID:SCR_012931

Protocol status: Working
We use this protocol and it's working
Add 25µl 2X precipitation buffer to 25µl of sample using a positive displacement pipet.

Vortex and let sit at RT for 10 minutes.

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

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

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.**

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**

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.
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.