MAY 14, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.ynsfvee

External link:
<https://mmpc.org/shared/document.aspx?id=93&docType=Protocol>

Protocol Citation: Peter Havel 2019. UC Davis - HDL Protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.ynsfvee>

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

Created: Feb 27, 2019

Last Modified: May 14, 2019

PROTOCOL integer ID:
 20914

UC Davis - HDL 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.

MATERIALS

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- ☒ 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**
- ☒ Platerreader **Contributed by users**

Note:

Fisher Scientific, [RRID:SCR_008452](#)
Abcam, [RRID:SCR_012931](#)

Keywords: HDL Protocol,
total cholesterol, triglyceride

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.