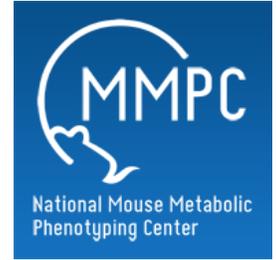


May 15, 2019

🌐 UC Davis - LDL Protocol

DOI

dx.doi.org/10.17504/protocols.io.yrcfv2w



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DOI: dx.doi.org/10.17504/protocols.io.yrcfv2w

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

Protocol Citation: Peter Havel 2019. UC Davis - LDL Protocol. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.yrcfv2w>

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Protocol status: Working

We use this protocol and it's working

Created: March 01, 2019

Last Modified: May 15, 2019

Protocol Integer ID: 20996

Keywords: cholesterol, triglyceride, LDL Protocol

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 25 μ l 2X precipitation buffer to 25 μ l of sample using a positive displacement pipet.
- 2 Vortex and let sit at RT for 10 minutes.
- 3 Centrifuge at 2000 \times g for 10 minutes at 4°C.
- 4 Pipet supernatant into new tube, this is the HDL fraction.
- 5 Add 5 μ l 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 μ l 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 5 μ l sample and 300 μ l PBS

- 7 Subtract blank readings from final readings. The assay will be linear so the unknown samples can be calculated as (Sample Absorbance \div Calibrator Absorbance) \times Calibrator Concentration.
- 8 HDL samples are diluted $\frac{1}{2}$ 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.