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UC Davis - β hydroxy butyrate Protocol

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OPEN ACCESS

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

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PROTOCOL integer ID:
21154

ABSTRACT

Summary:

When a sample is mixed with R1, AcAc in the sample is broken down to acetone by AADC. Upon addition of R2, 3-HB in the sample is oxidized in the presence of 3-HBDH and Thio-NAD. This oxidation triggers the cyclic reactions. Since the original AcAc in the sample has been removed, only 3-HB is assayed by measuring the rate of Thio-NADH production spectrophotometrically.

MATERIALS

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Calibrator **FUJIFILM Wako Diagnostic U.S.A. Catalog #412-73791**

Reagents **FUJIFILM Wako Diagnostic U.S.A. Catalog #417-73501, 413-73601**

Microplate **Contributed by users**

Platereader **Contributed by users**

Reagent Preparation:

R1 – reconstitute with buffer provided

R2 – reconstitute with buffer provided

Note:

FUJIFILM Wako [RRID:SCR_013651](https://www.fujifilm.com/usa/research/chemicals/assay-reagents/3-hydroxybutyrate-coa-assay-kit/)

Keywords: β hydroxy
butyrate

- 1 Reconstitute R1 and R2 using the buffers provided.
- 2 Add 4 μ l of calibrator and sample to each well.
- 3 Add 270 μ l of R1 to each well. Incubate at 37°C for 5 minutes.

IMPORTANT: Make sure not to add any bubbles to the wells when dispensing reagents, this will interfere with reading in the platereader.

- 4 Add 90 μ l of R2 to each well. Incubate at 37°C for 2 minutes. Read at 405 nm. Then continue reading every 30 seconds for 2 minutes.
- 5 Calculate the slope of the reaction for each well. The assay will be linear so the unknown samples can be calculated as $(\text{Sample } \Delta\text{OD}/\text{min} \div \text{Calibrator } \Delta\text{OD}/\text{min}) \times \text{Calibrator Concentration}$.