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## UC Davis - $\beta$ hydroxy butyrate Protocol

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:**  $\beta$  hydroxy butyrate, hydroxy butyrate protocol summary, original acac in the sample, nadh production, acac in the sample, hb in the sample, acetone, acetone by aadc, nad, oxidation

## 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

### MATERIALS

 Calibrator **FUJIFILM Wako Pure Chemical Corporation Catalog #412-73791**

 Reagents **FUJIFILM Wako Pure Chemical Corporation Catalog #417-73501, 413-73601**

 Microplate

 Platereader

### Reagent Preparation:

R1 – reconstitute with buffer provided

R2 – reconstitute with buffer provided

### Note:

**FUJIFILM Wako [RRID:SCR\\_013651](#)**

## Troubleshooting



- 1 Reconstitute R1 and R2 using the buffers provided.
- 2 Add 4 µl of calibrator and sample to each well.
- 3 Add 270 µl 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 µl 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  $(\text{Sample } \Delta\text{OD}/\text{min} \div \text{Calibrator } \Delta\text{OD}/\text{min}) \times \text{Calibrator Concentration}$ .