UC Davis MMPC-Live Protocol
β-Hydroxybutyrate Assay

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Replaces version: None
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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 measureing the rate of Thio-NADH production spectrophotometrically.

Reagents and Materials:

Reagent/Material	*Vendor*	*Stock Number*
Calibrator	Wako	412-73791
Reagents	Wako	417-73501 413-73601
Microplate		
Platereader		

Protocol:

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 (Sample ΔOD/min ÷ Calibrator ΔOD/min) × Calibrator Concentration.

Reagent Preparation:

- R1 – reconstitute with buffer provided
- R2 – reconstitute with buffer provided