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Working

UC Davis - ß hydroxy butyrate Protocol

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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 measureing the rate of Thio-NADH production spectrophotometrically.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=90&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR
Calibrator	412-73791	FUJIFILM Wako Diagnostic U.S.A.
Reagents	417-73501, 413-73601	FUJIFILM Wako Diagnostic U.S.A.
Microplate		
Platereader		

MATERIALS TEXT

Reagent Preparation:

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

Note:
FUJIFILM Wako RRID:SCR_013651

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.