BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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β-Hydroxybutyrate (BHB) Assay Kit (Cat #: A-134)

COMPONENTS: BHB Assay Solution: 5 ml (100 wells); store at -80°C **(shield solution from light after thawing)**
10 mM BHB Standard: 0.5 ml; store at -80°C
PEG Solution- 3 ml, **store at room temperature (RT**; **agitate vial to redissolve contents upon arrival)**

PRODUCT DESCRIPTION: The BHB Assay Kit is based on reduction of the tetrazolium salt INT in a NADH-coupled enzymatic reaction to formazan, which exhibits an absorption maximum at 492 nm, and can measure β-hydroxybutyrate in the cell and released to the culture medium, circulation, or urine in a quantitative manner. Reagents are stable for at least one year if stored and handled properly.

PROTOCOLS

Preparation of serum and cell culture medium: These samples are deproteinized by PEG precipitation. Mix 25 µl of each sample with 25 µl PEG Solution in a 1.5-ml microtube (PEG solution should be pipetted slowly using a cut tip). Vigorously vortex tube to ensure thorough mixing. Keep tube on ice for 5 min. Centrifuge solution in a refrigerated microfuge at ~14,000 rpm for 5 min. Harvest supernatant and store at -20°C.

Preparation of tissue/cell samples: Cell and tissue samples are deproteinized by PEG precipitation. Please follow the extraction protocol at https://bmrservice.com/deproteination-by-peg. Alternatively, the samples can be deproteinized by TCA precipitation (https://bmrservice.com/deproteination-by-tca). Deproteinized samples may be diluted with dH_2O prior to assay to achieve optimal result.

Urine: Urine samples can be assayed directly without pretreatment.

β-hydroxybutyrate (BHB) standard: Dilute the 10 mM BHB standard 10-fold with dH_2O to 1 mM (1000 µM), e.g. 450 µl dH_2O + 50 µl 10 mM BHB. Perform additional dilution with dH_2O to generate 500 µM, 250 µM and 125 µM BHB. Store diluted standards at -80°C.

ASSAY:

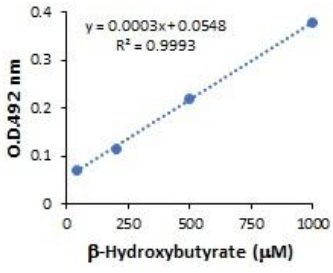
1. Thaw BHB Assay Solution and BHB standard quickly and keep solutions on ice. Do not over thaw solutions. Shield BHB Assay Solution from light during assay. Freeze solutions immediately after use.

2. Add 20 µl of each BHB standard and sample to a plain (uncoated) 96-well microplate.

3. Gently agitate BHB Assay Solution before first pipetting. Reaction is initiated by addition of 50 µl BHB Assay Solution to each standard and sample well. Mix contents by gentle agitation for ~10 sec. Cover plate and incubate at 37°C for 30 - 60 min. *Do not use CO_2 incubator.*

4. Eliminate air bubbles present in wells if necessary. Measure O.D. at 492 nm using a plate reader.

5. Plot BHB standards vs. O.D.492 nm. Generate a trend line equation on chart. Calculate sample BHB concentration using the derived equation (x = sample BHB concentration in µM; y = sample O.D.492 nm). A new plot must be generated for each assay.



ADDITIONAL INFORMATION

- BHB Assay Solution contains the organic solvent DMSO and iodonitrotetrazolium violet. Please contact us or visit the product webpage for material SDS information.
- Agitate PEG Solution vial in warm water to re-dissolve PEG precipitates if necessary.