BIOMEDICAL RESEARCH SERVICE, UNIVERSITY AT BUFFALO

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β-Hydroxybutyrate Dehydrogenase (BDH) Assay Kit (Cat #: E-139)

COMPONENTS: BDH Assay Solution- 5 ml (for 100 wells), store at -80ºC **(shield solution from light during assay)**
10x BDH Substrate- 0.5 ml, store at -20°C or -80°C
10x Cell Lysis Solution- 25 ml, store at room temperature **(agitate vial to redissolve contents)**

PRODUCT DESCRIPTION: The BDH enzyme activity assay is based on the reduction of INT in a NADH-coupled reaction to formazan, which exhibits an absorption maximum at 492 nm (ε = 18 $mM^{-1}cm^{-1}$) and allows for measurement of BDH activity in tissue/cell extracts. Reagents are stable for several years if stored and handled properly.

Preparation of cell/tissue extracts:

1. Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH_2O. Bring up ~10^5 washed cells in 50 – 100 µl ice-cold 1x Cell Lysis Solution by pipetting up and down gently. Leave lysate on ice for 5 min with agitation. If lysate is overly turbid, add more 1x Cell Lysis Solution and repeat pipetting. Tissue is homogenized in ice-cold 1x Cell Lysis Solution (~10 mg tissue in 0.5 ml).
2. Centrifuge lysate in a cold microfuge at ~14,000 rpm for 5 min. Supernatant is harvested and stored at -80ºC.
3. Use the BCA protein assay method to determine lysate protein concentration. A suggested sample protein concentration range is 0.5 – 3 mg/ml. *Normalization of lysate protein concentration is recommended.*

Reagent thawing:

Thaw samples, BDH Assay Solution, and 10x BDH Substrate quickly and keep on ice. Do not over thaw. Keep thawed BDH Assay Solution shielded from light. Gently agitate assay solution prior to first pipetting. It is important to minimize the time the reagents are thawed. Freeze solutions immediately after use.

Preparation of control solution and reaction solution:

Control solution is prepared by mixing 1 part of dH_2O and 9 parts of BDH Assay Solution, e.g. 50 µl dH_2O mixed with 450 µl BDH Assay Solution. Keep solution on ice.

Reaction solution is prepared by mixing 1 part of 10x BDH Substrate and 9 parts of BDH Assay Solution, e.g. 50 µl 10x BDH Substrate mixed with 450 µl BDH Assay Solution. Keep solution on ice.

Enzyme assay:

1. Each sample will be treated with 50 µl control solution and 50 µl reaction solution. Add 20 µl of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction).
2. After all samples have been pipetted to the plate in duplicate, swiftly add 50 µl control solution to one set of wells and 50 µl reaction solution to the other set of wells. Mix contents by gentle agitation for 10 sec. Cover plate and incubate in a 37°C humidified incubator for 60 or 120 min. *Do not use CO_2 incubator.* Cherry red color should gradually appear in wells.
3. Remove air bubbles from wells if necessary. Measure $O.D._{492\ nm}$ using a plate reader.
4. Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (**ΔO.D.**) for enzyme activity calculation. If incubation for 60 min, sample BDH activity in IU/L = µmol/(L•min) = **ΔO.D.** × 1000 × 70 µl / (60 min × 0.4 cm × 18 × 20 µl) = **ΔO.D. × 8.1**. If incubation for 120 min, BDH activity = **ΔO.D. x 4.05**. Enzyme activity may be normalized by GAPDH enzyme activity (see cat#E-101) or protein concentration.

Additional information:

- The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact us for SDS information.
- 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.