



TECHNICAL MANUAL

β-Hydroxybutyrate Dehydrogenase (BDH) Activity Assay Kit

- **SKU CODE:** BR00004
- **SIZE:** 100 wells
- **DETECTION PRINCIPLE:** Colorimetric
- **RUO:** Research-Use-Only

β -Hydroxybutyrate Dehydrogenase (BDH) Activity Assay Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Specification:

100 wells

Measuring instrument:

Microplate reader (O.D._{492 nm})

Sample type:

Cell/tissue lysates and serum

2. Storage & Expiry

This product should be stored according to the conditions listed for each kit component. Reagents are stable for several years if stored and handled properly. For detailed storage instructions of individual kit components, please refer to Section 4. The expiration date is indicated on the outer label of the kit box.

3. 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 ($\epsilon = 18 \text{ mM}^{-1}\text{cm}^{-1}$) and allows for measurement of BDH activity in cell/tissue lysates and serum samples.

4. Kit Contents

No	Component Name	Size	Storage
1	BDH Assay Solution	5 ml (for 100 wells)	-80°C (shield solution from light during assay)
2	10x BDH Substrate	0.5 ml	-20°C (short term) or -80°C (long term)
3	10x Cell Lysis Solution	25 ml	Room temperature (agitate vial to redissolve contents)

Note: All reagents must be stored according to the specified conditions listed in the table above. Do not mix reagents from different kits, as this may compromise assay performance. For reagents provided in small volumes, centrifuge briefly before use to ensure complete recovery of the contents.

Additional materials required:

1. 37°C humidified incubator (not a CO₂ incubator).
2. Microplate Reader (492 nm).
3. Plain (uncoated) 96-well plate.
4. Refrigerated microcentrifuge (~14,000 rpm).
5. Precision pipettes and disposable pipette tips. Distilled water.
6. Disposable tubes for sample dilution.
7. BCA protein assay reagents.

5. Important Notes

1. This assay kit is intended for Research Use Only. Assay Genie assumes no responsibility for any issues or legal liabilities arising from the use of this kit for clinical diagnostics or any other unauthorized purposes.
2. Please read the instructions carefully before beginning the assay. Ensure that all instruments are correctly calibrated. Strict adherence to the protocol is essential for accurate results.
3. Appropriate laboratory safety precautions must be followed, including the use of lab coats and latex gloves.
4. If the concentration of the target substance falls outside the detection range, please adjust the sample by performing further dilution or concentration as needed.
5. If your sample type is not listed in the instruction manual, we strongly recommend performing a preliminary test to confirm compatibility.
6. Experimental outcomes depend on multiple factors including reagent integrity, handling technique, and laboratory conditions. While Assay Genie guarantees the quality of our kits, we are not responsible for any loss of samples during use. We

advise calculating sample requirements in advance and ensuring adequate sample volume is reserved before starting the assay.

7. The assay solution contains dimethyl sulfoxide (DMSO) and iodonitrotetrazolium (INT). Please refer to the product webpage or contact Assay Genie for SDS information.
8. 10x Cell Lysis Solution precipitates at cold temperature. Re-dissolve contents by agitation in warm water upon arrival.

6. Reagent Preparation

1. **1x Cell Lysis Solution:** Prepare 1x Cell Lysis Solution by diluting 10x Cell Lysis Solution with dH₂O.
2. **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.
3. **Control solution:** Prepared by mixing 1 part of dH₂O and 9 parts of BDH Assay Solution, e.g. 50 µl dH₂O mixed with 450 µl BDH Assay Solution. Keep solution on ice.
4. **Reaction solution:** 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.

Item	Control solution	Reaction solution
dH ₂ O	50 µl	–
10x BDH Substrate	–	50 µl
BDH Assay Solution	450 µl	450 µl

7. Sample Preparation

A. Sample Preparation

Cell/tissue samples

1. Bring up $\sim 10^5$ washed cells in 50 – 100 μ l 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 $\sim 14,000$ rpm for 5 min. Supernatant is harvested and stored at -80°C .
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.

Serum samples

1. Dilute serum 10-fold with ice-cold dH_2O prior to use. Do not use plasma for the assay.

B. Dilution

Sample Type	Dilution factor
Serum	10-fold

Note: The recommended diluent is ice-cold dH_2O . For sample types not specified in the protocol, it is advised to perform a preliminary test to determine the appropriate dilution factor or contact our Tech Support Team at techsupport@assaygenie.com.

8. Assay Procedure

1. **Add samples:** Each sample will be treated with 50 μ l control solution and 50 μ l reaction solution. Add 20 μ l of each sample to duplicate wells of a plain (uncoated) 96-well plate (for control and reaction).

2. **Add solutions and incubate:** After all samples have been pipetted to the plate in duplicate, swiftly add 50 μ l control solution to one set of wells and 50 μ l 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 CO₂ incubator. Cherry red color should gradually appear in wells.
3. **Measure:** Remove air bubbles from wells if necessary. Measure O.D._{492 nm} using a plate reader.
4. **Calculate Δ O.D.:** Subtract control well reading from reaction well reading for each sample. Use the subtracted reading (Δ O.D.) for enzyme activity calculation.

9. Data Analysis

Calculation:

If incubation for 60 min, sample BDH activity in IU/L = $\mu\text{mol}/(\text{L} \cdot \text{min}) = \Delta\text{O.D.} \times 1000 \times 70 \mu\text{l} / (60 \text{ min} \times 0.4 \text{ cm} \times 18 \times 20 \mu\text{l}) = \Delta\text{O.D.} \times 8.1$

If incubation for 120 min, BDH activity = $\Delta\text{O.D.} \times 4.05$

Enzyme activity may be normalized by GAPDH enzyme activity or protein concentration.

Note:

Δ O.D.: reaction well reading minus control well reading at 492 nm.

70 μ l: total volume per well (20 μ l sample + 50 μ l control or reaction solution).

min: incubation time.

0.4 cm: light path length.

18: extinction coefficient of formazan at 492 nm ($\text{mM}^{-1}\text{cm}^{-1}$).

20 μ l: sample volume.

1000: conversion factor from mmol to μmol .

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