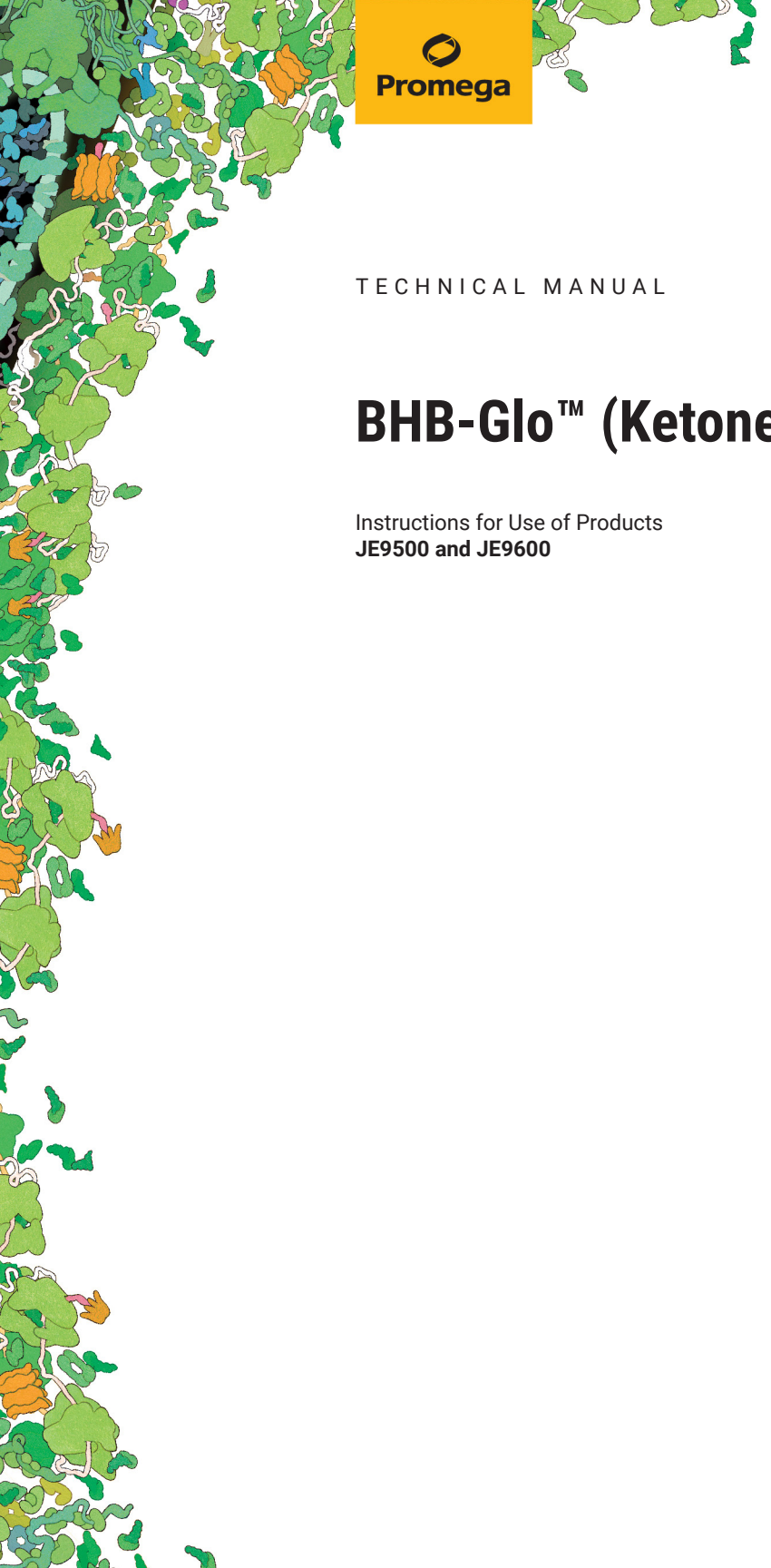

TECHNICAL MANUAL

BHB-Glo™ (Ketone Body) Assay

Instructions for Use of Products
JE9500 and JE9600

Revised 1/24
TM713

BHB-Glo™ (Ketone Body) Assay

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The BHB-Glo™ (Ketone Body) Assay is a bioluminescent assay for rapid, selective, sensitive detection of β-hydroxybutyrate (BHB) in biological samples. β-hydroxybutyrate is a ketone body that is synthesized in the liver from fatty acids during low glucose conditions, such as starvation or prolonged exercise. BHB acts as an energy source for peripheral tissues and is believed to have signaling properties that involve many cellular functions.

The BHB-Glo™ (Ketone Body) Assay couples BHB oxidation and NADH production with a bioluminescent NADH detection system (Figure 1; 1–3). When BHB detection reagent is added to a sample at a 1:1 ratio, the coupled-enzyme reactions are initiated and run simultaneously (Figure 2). The luminescent signal is proportional to the amount of BHB in the sample and increases until all BHB is consumed, at which point a stable luminescent signal is achieved (Figure 3 and Table 1).

The BHB-Glo™ (Ketone Body) Assay sensitivity is <40nM (20pmol of BHB in a 50µl sample) with linearity up to 25µM. The BHB-Glo™ Assay is versatile and compatible with many sample types, including cell culture medium, mammalian cells, tissues and serum. However, similar to other enzyme-coupled BHB detection methods, enzymes and reduced dinucleotides in samples, such as NAD(P)H, can interfere with the assay. We recommend up-front sample preparation to inhibit endogenous enzyme activity and degrade NAD(P)H. To simplify sample preparation, we provide a protocol that uses a strong acid to lyse samples, inactivate enzymes and degrade NAD(P)H. The workflow is compatible with 96- and 384-well plate formats, does not require sample centrifugation or spin columns and is well suited for rapidly analyzing multiple samples.

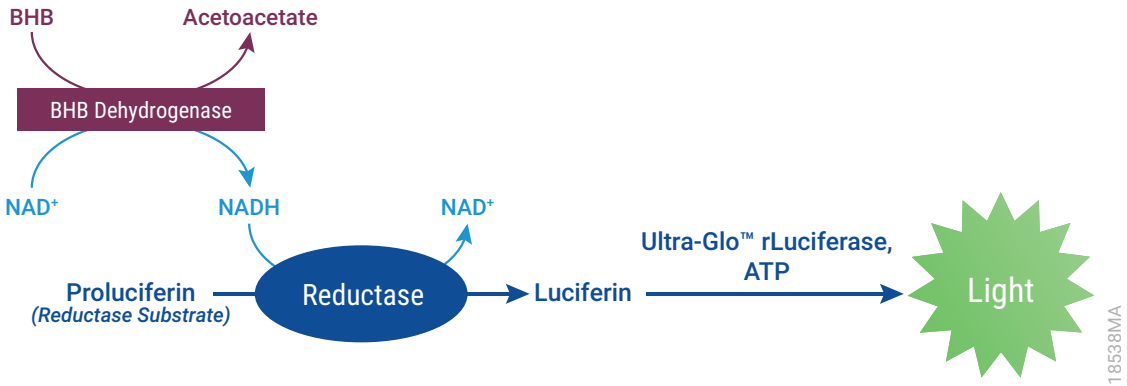

Figure 1. Schematic diagram of the BHB-Glo™ (Ketone Body) Assay principle. β-Hydroxybutyrate (BHB) Dehydrogenase catalyzes the oxidation of BHB with concomitant reduction of NAD+ to NADH. In the presence of NADH, reductase enzymatically reduces a proluciferin Reductase Substrate to luciferin. Luciferin is detected using Ultra-Glo™ Recombinant Luciferase and the amount of light produced is proportional to the amount of BHB in the sample.

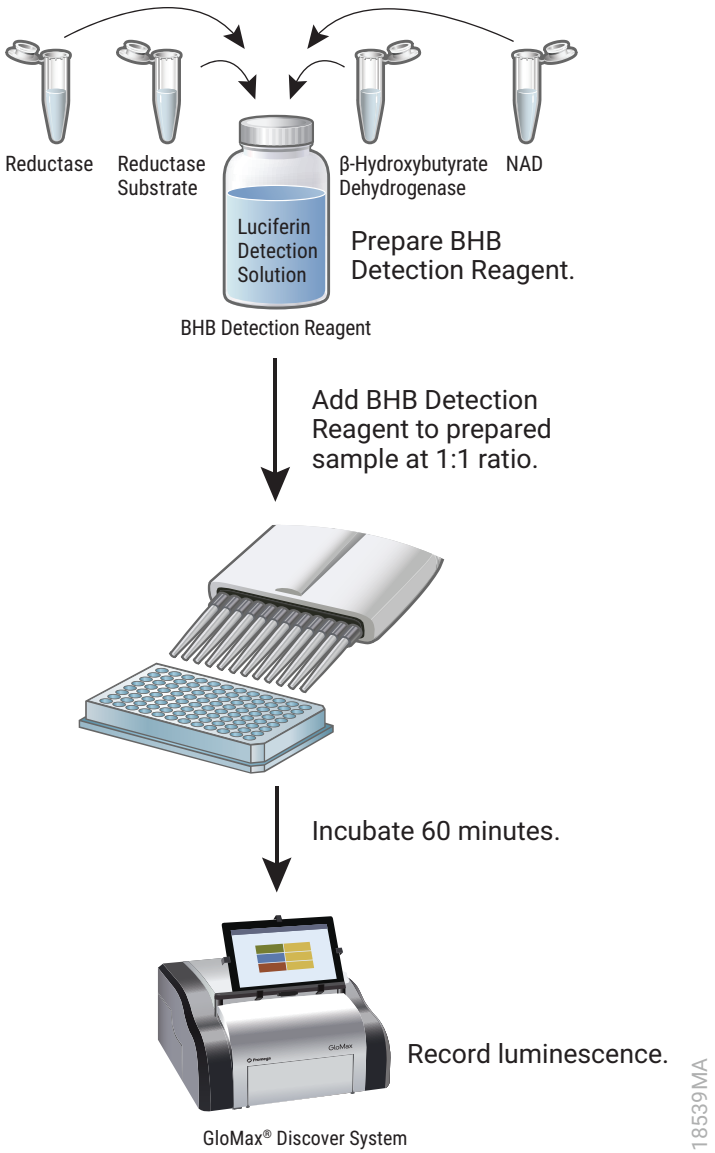

Figure 2. BHB-Glo™ (Ketone Body) Assay reagent preparation and protocol. BHB Dehydrogenase is β-Hydroxybutyrate Dehydrogenase.

1. Description (continued)

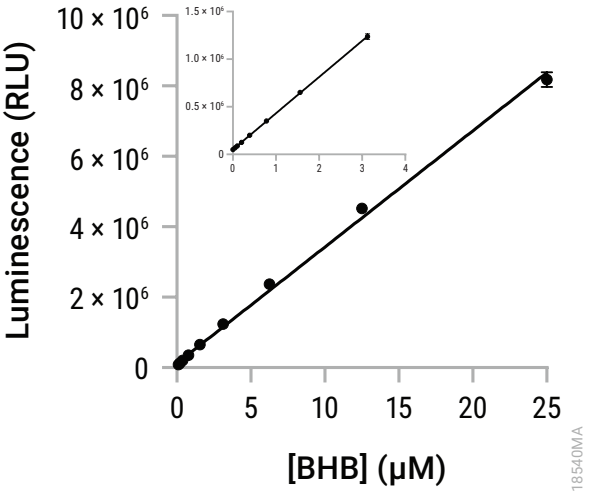

Figure 3. BHB titration curve. Twofold serial dilutions of BHB in the range of 25µM to 40nM were prepared in phosphate-buffered saline (PBS). The negative control was PBS containing no BHB. Aliquots of the prepared dilutions (50µl) were transferred to a 96-well plate and the BHB-Glo™ (Ketone Body) Assay was performed following the protocol in Section 3.B. Luminescence was measured in relative light units (RLU) using a GloMax® Discover System (Cat.# GM3000). Each data point represents the average of three replicates.

Table 1. BHB Titration Data. Signal-to-background ratio (S/B) was calculated by dividing mean luminescence for samples by the mean luminescence for the negative control (no BHB). Signal-to-noise ratio (S/N) was calculated by dividing net luminescence (mean luminescence for the sample minus mean luminescence for the negative controls) by the standard deviation of the negative control.

BHB (µM)	0	0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25
Average Luminescence (RLU × 10^3)	51	70	88	125	201	351	650	1,237	2,367	4,524	8,183
Standard Deviation (RLU × 10^3)	4.0	4.1	4.6	5.2	7.3	10.4	14.6	28.6	57.7	134	210
Coefficient of Variation (%)	8	6	5	4	4	3	2	2	2	3	3
S/B	1.0	1.4	1.7	2.4	4.0	6.9	12.9	24.5	46.8	89.5	162
S/N	—	4.7	9.2	18.5	37.2	74.2	148	293	573	1,106	2,011

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
BHB-Glo™ (Ketone Body) Assay	**5ml**	**JE9500**

The system contains sufficient reagents to perform 100 reactions in 96-well plates. Includes:

Metabolite-Glo™ Detection System, 5ml:

- 5ml Luciferin Detection Solution
- 55µl Reductase
- 55µl Reductase Substrate
- 275µl NAD
- 100µl NADP*
- 15ml 0.6N HCl
- 15ml Neutralization Buffer

BHB-Glo™ (Ketone Body) Enzyme Pack, 5ml:

- 100µl β-Hydroxybutyrate Dehydrogenase
- 50µl β-Hydroxybutyrate, 10mM

PRODUCT	SIZE	CAT.#
BHB-Glo™ (Ketone Body) Assay	**50ml**	**JE9600**

The system contains sufficient reagents to perform 1,000 reactions in 96-well plates. Includes:

Metabolite-Glo™ Detection System, 50ml:

- 50ml Luciferin Detection Solution
- 275µl Reductase
- 275µl Reductase Substrate
- 1ml NAD
- 0.5ml NADP*
- 15ml 0.6N HCl
- 15ml Neutralization Buffer

BHB-Glo™ (Ketone Body) Enzyme Pack, 50ml:

- 1ml β-Hydroxybutyrate Dehydrogenase
- 50µl β-Hydroxybutyrate, 10mM

Storage Conditions: Store the BHB-Glo™ (Ketone Body) Assay at less than –65°C. Alternatively, store the Reductase Substrate at less than –65°C protected from light and all other components at –30°C to –10°C, except the 0.6N HCl and Neutralization Buffer, which can be stored at +2°C to +10°C or at room temperature. Do not freeze-thaw the kit components more than three times. As needed, dispense kit components into single-use aliquots to minimize freeze-thaw cycles.

*NADP is a component of the Metabolite-Glo™ Detection System, but is not used when performing the BHB-Glo™ Assay.

Use personal protective equipment and follow your institution's safety guidelines and disposal requirements when working with biohazardous materials such as cells and cell culture reagents.

3. Measuring BHB

Materials to Be Supplied By the User

- phosphate-buffered saline (PBS, e.g., Sigma Cat.# D8537 or GIBCO™ Cat.# 14190)
- 96-well assay plates (opaque white-walled or clear bottom, e.g., Corning® Cat.# 3903 or 3912)
- luminometer (e.g., GloMax® Discover System, Cat.# GM3000)

3.A. Sample Preparation

! Metabolism is a dynamic process. Work quickly when collecting and preparing samples.

The BHB-Glo™ Assay can be used to measure BHB in samples such as cell medium, mammalian cells, tissues and serum. This requires preparing various sample types, including cell lysates and tissue homogenates, before assaying.

For sample preparation, we recommend using 0.6N HCl (acid) and Neutralization Buffer (1M Tris base) supplied with the kit. Acid treatment rapidly stops metabolism, inhibits endogenous protein activity and destroys reduced NAD(P)H dinucleotides. When dealing with difficult-to-lyse samples such as 3D cultures, Triton®X-100 can be added to a final concentration of 0.2%. Acid-treated and neutralized samples can be assayed immediately following the protocol provided in Section 3.B or stored at –20°C. If needed, an aliquot of the sample can be removed for protein measurement.

We also do not recommend using detergent lysis without acid since many dehydrogenases remain active in detergent lysed samples, significantly increasing the BHB-Glo™ Assay background. Endogenous dehydrogenase activity can be determined by using BHB detection reagent prepared without β-Hydroxybutyrate Dehydrogenase. If, in the absence of β-Hydroxybutyrate Dehydrogenase, sample luminescence signal is higher compared to assay background (buffer only) control, sample deproteinization/enzyme inactivation is required.

Acid lysis is compatible with plate workflow and can be directly added to the cells in 96- or 384-well plates. Acid treatment can be used with different sample types and is highly recommended for unknown samples. Table 2 provides examples of BHB concentration ranges in samples and suggestions for sample preparation. Section 4 provides example protocols for using the BHB-Glo™ Assay with cell media, mammalian cells, tissues and serum.

Samples prepared using other methods, for example deproteinized using 10kDa filtration columns or heat inactivation, might be acceptable but must be tested for compatibility with the BHB-Glo™ Assay using the provided BHB standards and controls described in Section 3.D.

! Perchloric acid or KOH treatment recommended by other kits is not compatible with the BHB-Glo™ Assay and should not be used.

Table 2. Recommended Sample Preparation. No significant difference in BHB concentration was measured in samples treated with 0.6N HCl between 1/5 to 1/2 of the sample volume. Therefore, different ratios can be used to accommodate your experimental setup. Concentrations in this table represent experimental values shown in this Technical Manual. Optimization may be required for other sample types or sources.

Sample	BHB Concentration in Sample	Recommendations
Cell medium (extracellular)	2–10µM for 50,000 HepG2 cells	• Cells in medium without FBS for 0–24 hours. • Add 0.6N HCl (1/5 to 1/2 of the sample volume). • Add Neutralization Buffer (the same volume as 0.6N HCl).
Cell lysates (intracellular)	0.04–0.2µM for 37,500 cells lysed in 50µl	• Cells in PBS. • Add 0.6N HCl (1/5 to 1/2 of the sample volume). • Add Neutralization Buffer (the same volume as 0.6N HCl).
Tissues	0.5–5µM for 10mg of wet tissue homogenized in 1ml	• Tissues in PBS. • Add 0.6N HCl (1/5 to 1/2 of the sample volume). • Add Neutralization Buffer (the same volume as 0.6N HCl).
Serum	100–500µM depending on human serum lot (varies)	• Serum samples diluted 40- to 60-fold in PBS. • Add 0.6N HCl (1/5 to 1/2 of the sample volume). • Add Neutralization Buffer (the same volume as 0.6N HCl).

3.B. Reagent Preparation

This protocol is for 50µl of sample and 50µl of BHB detection reagent in a 96-well plate. The assay can be adapted to other volumes provided the 1:1 ratio of BHB detection reagent volume to sample volume is maintained (e.g., 12.5µl of sample and 12.5µl of BHB detection reagent in a 384-well plate format). To use a different assay format, scale the volume of samples, controls, BHB standards and reagents accordingly.

1. Thaw all components on ice or at room temperature. Once thawed, equilibrate the Luciferin Detection Solution to room temperature; place all other components on ice. Mix thawed components prior to use.
2. Calculate the volume of BHB detection reagent needed. You will need 50µl of BHB detection reagent for each assay in a 96-well plate with 50µl of sample. We recommend preparing additional reagent to compensate for pipetting error.

3.B. Reagent Preparation (continued)

3. Prepare BHB detection reagent by combining components as shown below.

   Note: Prepare only the volume of BHB detection reagent calculated in Step 2. Unused BHB detection reagent cannot be stored.

Component	Volume Per Reaction	Volume Per 100 Reactions
Luciferin Detection Solution	50µl	5ml
Reductase Substrate	0.25µl	25µl
Reductase	0.25µl	25µl
NAD	0.25µl	25µl
β-Hydroxybutyrate Dehydrogenase	1µl	100µl

4. Mix by gently inverting five times.

3.C. Protocol

When performing the BHB-Glo™ Assay, be sure to use assay plates that are compatible with your luminometer. See Section 5.B for more information.

Information on preparing and using appropriate positive and negative controls for the BHB-Glo™ Assay can be found in Section 3.D.

1. Prepare samples using the appropriate method for your sample type. See Sections 3.A and 4 for more information.
2. Prepare the BHB detection reagent as described in Section 3.B. Ensure that the reagent is at room temperature prior to use.
3. Transfer 50µl of each sample, positive controls (BHB standards diluted in the same buffer as the samples) and negative (buffer only) controls into a well of an opaque white 96-well plate.
4. Add 50µl of BHB detection reagent to each well.
5. Mix the plate by shaking for 30–60 seconds.
6. Incubate for 60 minutes at room temperature.

   Note: The light signal continues to increase until all BHB is consumed and the signal plateaus. At any time point the signal is directly proportional to the BHB concentration.
7. Record luminescence using a plate-reading luminometer, following the instrument manufacturer's instructions (Section 5.B).

3.D. Assay Controls and Data Analysis

There is a linear relationship between luminescence signal and BHB concentration and many luminescent measurements can be described simply in terms of relative light units (RLU). The data can be analyzed as the change in RLU values between the experimental controls and test conditions. When comparing changes in luminescence, wells containing buffer only should be included as negative controls and can be subtracted as assay background.

To calculate BHB concentration and determine if your samples are within the linear range of the assay, a standard curve using a titration of β-Hydroxybutyrate, 10mM, included in the kit, can be used (see Table 1 for suggested concentrations). If the sample RLU values fall outside the linear range of the BHB standard curves, sample dilutions should be adjusted and reassayed.

Alternatively, instead of running a full standard curve, 2–4 concentrations of BHB standard can be used and the concentration of BHB in samples calculated based on RLU from those standard concentrations. We recommend including a high (25µM) and low concentration (1–2µM) of BHB. These amounts can be adjusted based on concentrations expected in the sample. To determine assay background, a negative control (buffer only) should be included on the assay plate.

BHB concentration in the sample can be calculated using the following formula if using one BHB standard concentration:

$$[\text{BHB}] = \frac{[\text{BHB standard}]\ (\mu\text{M}) \times (\text{RLU}_{\text{sample}} - \text{RLU}_{\text{background}})}{(\text{RLU}_{\text{BHB standard}} - \text{RLU}_{\text{background}})}$$

To determine if samples contain NAD(P)H or other interfering substances that increase the background signal, samples can be assayed with BHB detection reagent without β-Hydroxybutyrate Dehydrogenase.

4. Example Protocols and Data for Various Sample Types

This section includes example protocols that were used to generate data depicted in this Technical Manual. Optimization may be required, depending on sample type and experimental conditions.

4.A. Cell Culture Medium

Metabolism is a dynamic process guided by fuel availability. The formulations of commonly used cell culture media, such as DMEM and RPMI 1640, contain different amounts of small metabolites, including glucose, glutamine, amino acids and other components, and should be considered when studying metabolism. Supplementing the culture medium with 5–10% fetal bovine serum (FBS) is a standard practice when culturing mammalian cells. However, FBS also contains variable amounts of metabolites. Using defined medium, for example DMEM (GIBCO™ Cat.# A1443001) lacking major fuel sources such as glucose, glutamine and pyruvate, adding those components at the desired concentrations, and then supplementing with dialyzed serum (e.g., GIBCO™ Cat.# A3382001), provides better control when studying metabolic changes.

4.A. Cell Culture Medium (continued)

The BHB-Glo™ (Ketone Body) Assay can be used for monitoring changes in extracellular BHB levels of cells plated in 96- or 384-well plates.

1. Plate 50,000 cells in 96-well plates and adhere overnight.
2. Wash the cells three times with 200µl of cold PBS, then add 100µl of medium (without FBS).
   Note: BHB is present in FBS, resulting in higher background levels and may affect BHB secretion/detection.
3. Remove medium samples at desired times.
   Note: Samples may be assayed immediately or frozen at –10°C or below.
4. Add 25µl of medium to a 96-well plate. Include negative (buffer only) and positive (BHB standard) controls as described in Section 3.B.
5. Add 12.5µl of 0.6N HCl and mix by shaking the plate for 5 minutes.
6. Add 12.5µl of Neutralization buffer. Mix by shaking the plate for 30–60 seconds.
7. Add 50µl of BHB detection reagent, prepared as described in Section 3.B.
8. Mix by shaking the plate for 30–60 seconds.
9. Incubate for 60 minutes at room temperature.
10. Record luminescence.

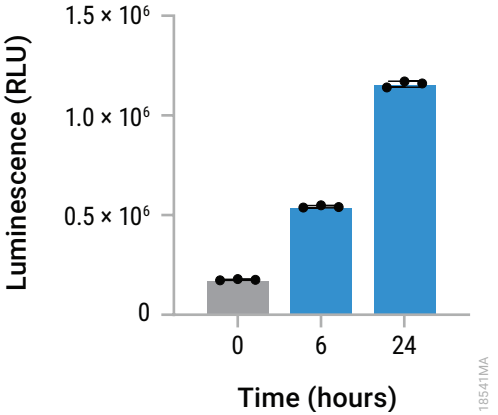

Figure 4. BHB secretion in cell medium. HepG2 cells were plated at 50,000 cells/well in a 96-well plate overnight in DMEM culture medium supplemented with 10% FBS. Cells were then washed three times with PBS to remove BHB from medium and supplemented serum. DMEM (GIBCO™ Cat.# A14430) without FBS + 5mM glucose + 2mM glutamine was added and aliquots of medium were removed after 6 and 24 hours. Medium was frozen below –10°C until assayed. Samples were thawed and BHB was measured as described in Section 4.A. The average luminescence for three replicates (indicated by dots) is shown in relative light units (RLU). The data show that the extracellular BHB was measured with >sixfold signal above background at 24 hours. Extracellular BHB levels increased from 3.1µM (6 hours) to 7.1µM (24 hours).

4.B. Collected or Suspension Mammalian Cells

The BHB-Glo™ Assay can be used for monitoring changes in intracellular BHB levels of cells collected, lysed and transferred to a 96- or 384-well plate. Alternatively, cells can be plated in a 96- or 384-well plate and BHB levels determined. Due to relatively low levels of intracellular BHB, higher cell numbers are required for measurement.

1. Collect cells, wash with cold PBS 3–4 times, and resuspend at a concentration of 0.8×10^5–3×10^6 cells/ml.
2. Add 1/2 volume of 0.6N HCl (e.g., add 500µl of 0.6N HCl per 1ml cells in PBS). Mix well and incubate for 5 minutes at room temperature.
3. Add the same volume of Neutralization Buffer as 0.6N HCl in Step 2. Mix well.

   Notes:

   a. Aliquots can be removed for protein measurements or samples can be stored below –20°C.

   b. One-fifth (1/5) volume of 0.6N HCl and Neutralization Buffer can be used to diminish protein assay interference without detriment to the BHB Assay.
4. Mix PBS + 0.6N HCl + Neutralization Buffer at 2:1:1 ratio (negative control, assay background). Prepare 1µM BHB standard, in the same buffer, as a positive control.
5. Transfer 50µl of cell lysates, negative controls and positive controls to the opaque white assay plate.

   Note: If cells need to be diluted, use buffer prepared in Step 4.
6. Add 50µl of BHB detection reagent, prepared as described in Section 3.B.
7. Mix by shaking the plate for 30–60 seconds.
8. Incubate for 60 minutes at room temperature.
9. Record luminescence.

4.B. Collected or Suspension Mammalian Cells (continued)

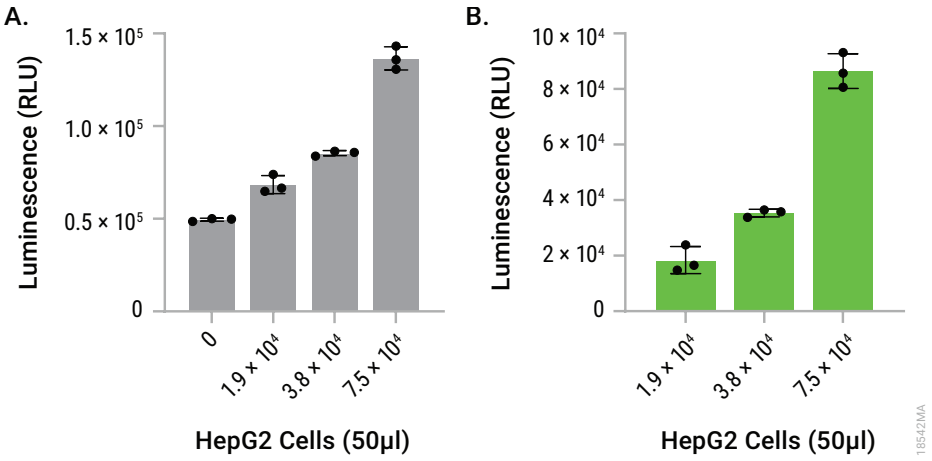

Figure 5. Intracellular BHB. Panel A. Data shown with background (no cells). **Panel B.** Data shown with background subtracted. HepG2 cells were collected and washed 4 times in cold PBS. 0.6N HCl (1/2 volume of PBS) was added for 5 minutes prior to addition of Neutralization Buffer (same volume as 0.6N HCl). Lysed and neutralized cells (50µl) were added at 18,750, 37,500 and 75,000 cells/well to a 96-well plate. Cell densities reflect twofold dilution with 0.6N HCl and Neutralization Buffer. BHB was measured as described in Section 4.B. The average luminescence for three replicates (indicated by dots) is shown in relative light units (RLU). The data show that the intracellular BHB was measured with >twofold signal above background with 75,000 HepG2 cells per well, depicting low intracellular BHB levels. Due to low intracellular BHB levels (~0.34 fmol/cell), the linear relationship between BHB concentration and cell number is confounded with background included (**Panel A**) but is more representative after background subtraction (**Panel B**).

4.C. Tissues

The BHB-Glo™ Assay can be used to measure the BHB concentration in homogenized tissues. To fit into the BHB-Glo™ Assay detection range and avoid assay interference, we recommend homogenizing the tissues at 5–15mg/ml of tissue in PBS with 0.6N HCl. Homogenized and neutralized tissues can be assayed immediately or stored below –10°C.

1. Weigh 5–15mg of tissue and add 1ml of PBS.
2. Add 200µl (1/5 sample volume) of 0.6N HCl and homogenize for 20–30 seconds using a mechanical homogenizer (e.g., Tissue-Tearor™, BioSpec Cat.# 985370-07).
3. Add 200µl of Neutralization Buffer (same volume as 0.6N HCl in Step 2) to homogenate from Step 2.

   Note: An aliquot of tissue homogenate can be removed at this point to determine protein concentration.
4. Transfer 50µl of each prepared sample into a 96-well assay plate. Include negative control (buffer only) and positive control (BHB standard). Prepare controls in the same buffer as samples (e.g., PBS + 0.6N HCl + Neutralization buffer at a 5:1:1 ratio).

5. Add 50µl of BHB detection reagent, prepared as described Section 3.B.
6. Mix by shaking the plate for 30–60 seconds.
7. Incubate at room temperature for 60 minutes.
8. Record luminescence.

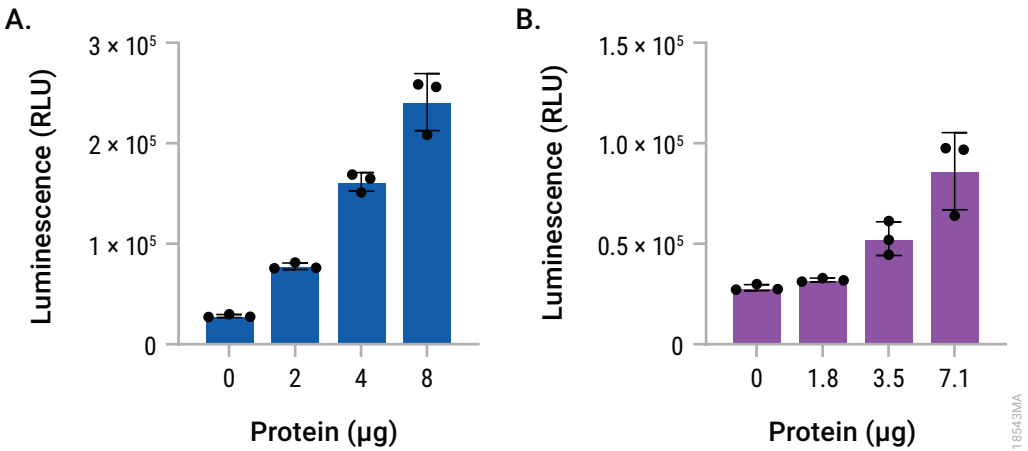

Figure 6. Measuring BHB in tissues. A sample (11mg/ml) of frozen mouse liver (**Panel A**) or brain (**Panel B**) tissue were homogenized as described in the protocol. An aliquot of each sample was removed for protein measurement. The protein concentration was 1.28mg/ml for liver (**Panel A**) and 1.14mg/ml for brain (**Panel B**). For BHB detection, samples were serially-diluted twofold in dilution buffer (PBS + 0.6N HCl + Neutralization Buffer premixed in a 5:1:1 ratio) and 50µl was transferred to the assay plate. Wells containing 50µl of homogenization buffer with and without 1µM BHB were included as the positive and negative controls, respectively. Fifty microliters (50µl) of BHB detection reagent was added, and after a 60-minute incubation at room temperature, luminescence was recorded.

4.D. Serum

The BHB-Glo™ (Ketone Body) Assay can be used to measure BHB in serum or plasma. Serum interferes with the BHB-Glo™ Assay and must be diluted prior to measuring BHB. The optimal dilution will vary depending on the serum source and BHB concentration. We recommend evaluating a series of serum dilutions to determine the dilution factor that results in sensitive detection of BHB with minimal interference. The sample interference can be tested by measuring a 'spike' recovery. A 'spike' is a known concentration of BHB standard (β-Hydroxybutyrate, 10mM, provided in kit) added to the sample. It is important when doing spike experiments to know the concentration of spike you are adding to each well, and to run the sample with and without spike while keeping all other well contents the same. Interference can be measured based on the recovery of the spike added relative to a BHB only control as shown in Figure 7. For minimal interference and optimal assay performance, serum samples should be diluted to achieve >80% spike recovery.

1. Make serum serial dilutions in PBS. Include a negative control (buffer only).
2. Prepare a 4µM BHB spike by diluting 10mM BHB standard (β-Hydroxybutyrate, 10mM) in PBS.
3. Combine diluted serum samples with either PBS (no spike) or 4µM BHB (spike) at a 1:1 ratio. Include positive (2µM BHB, final concentration) and negative control (buffer only).

   Note: If assay conditions are optimized to reduce interference, Steps 2 and 3 can be omitted.
4. Transfer 30µl of each prepared sample into a 96-well assay plate.
5. Add 10µl of 0.6N HCl. Mix by shaking the plate for 5 minutes.
6. Add 10µl of Neutralization Buffer. Mix by shaking the plate for 30–60 seconds.
7. Add 50µl BHB detection reagent prepared as described Section 3.B.
8. Mix by shaking the plate for 30–60 seconds.
9. Incubate at room temperature for 60 minutes.
10. Record luminescence.
11. Calculate percent recovery using this formula:

$$\text{Percent Recovery} = \frac{(RLU_{\text{serum + BHB spike}} - RLU_{\text{serum}})}{(RLU_{\text{BHB standard}})} \times 100$$

Note: After assay conditions are optimized to reduce interference, Steps 2, 3 and 11 can be omitted.

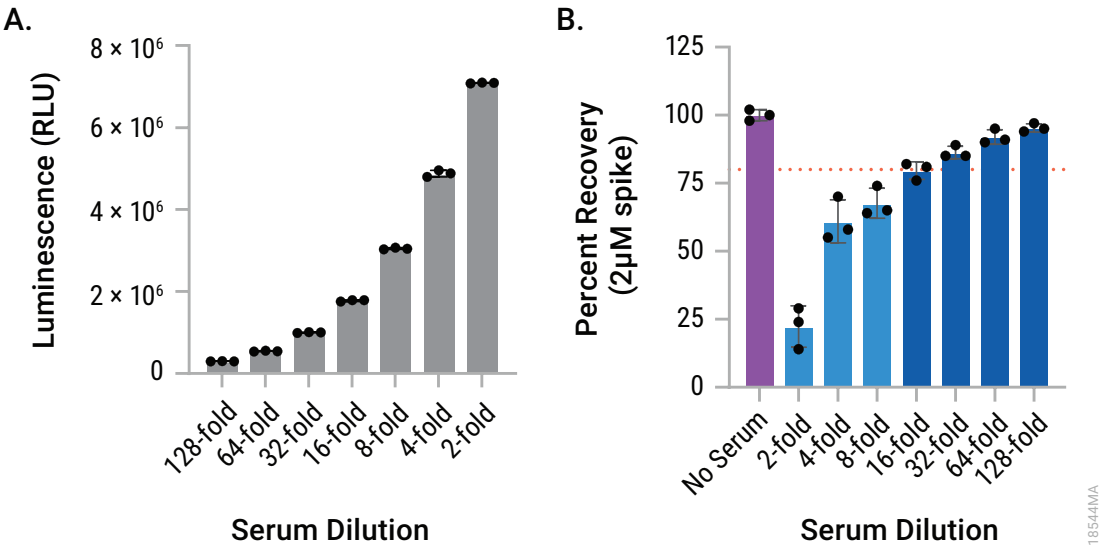

Figure 7. Optimization of BHB detection in serum. Serial dilutions of human serum were prepared in PBS (**Panel A**) or PBS mixed 1:1 with 4µM BHB standard for spike samples (**Panel B**). Dilutions were transferred to a white 96-well plate (30µl per well). Samples were acidified with 0.6N HCl (10µl per well; **Panels A** and **B**) for 5 minutes, then Neutralization Buffer was added (10µl per well). Percent recovery, shown in **Panel B**, was calculated as described in the protocol. Treating serum with acid improved the ability to measure BHB in less dilute samples (**Panel B**). In acid-treated samples (**Panels A** and **B**), >80% recovery (dotted line) was achieved with 16-fold diluted samples and BHB detection was linear with samples diluted from 16- to 128-fold. In 128-fold diluted serum, BHB was measured with approximately tenfold signal above background (~100µM), indicating that BHB concentration in serum can be measured with high sensitivity and wide assay window. Luminescence was measured on a GloMax® Discover System. Data is the average of 3 replicates.

5. Appendix

5.A. Temperature and Reagent Compatibility

The intensity and stability of the luminescent signal is temperature sensitive. For consistent results, equilibrate the reagents to room temperature before using.

Avoid the presence of DTT and other reducing agents in the samples to be tested. Reducing agents will react with the Reductase Substrate and increase background.

5.B. Plates and Equipment

Most standard plate readers are designed to measure luminescence and are suitable for this assay. Some instruments do not require gain adjustment while others might require optimizing the gain settings to achieve sensitivity and dynamic range. An integration time of 0.25–1 second per well should serve as a guidance. For exact instrument settings consult the instrument manual.

Use opaque, white multiwell plates that are compatible with your luminometer (e.g., Corning® Costar® 96-well plates, Cat.# 3917; Costar® 384-well plates, Cat.# 3570). For cultured cells, opaque white-walled clear bottom tissue culture plates (e.g., Corning® 96-well plates, Cat.# 3903) are acceptable. Luminescent metabolite assays are well suited for miniaturization. When samples are limited, consider using 96-well half-area (Corning® Cat.# 3696), 384-well (Costar® Cat.# 3570) or 384-well low volume (Corning® Cat.# 4512) plates. We do not recommend black or clear plates. Light signal is diminished in black plates and increased well-to-well crosstalk is observed in clear plates.

Note: The RLU values shown in the figures of this Technical Manual vary, depending on the plates and luminometers used to generate data. Although relative luminescence output will vary with different instruments, this variation does not affect assay performance.

5.C. References

1. Zhou, W. *et al.* (2014) Self-immolative bioluminogenic quinone luciferins for NAD(P)H assays and reducing capacity-based cell viability assays. *Chembiochem.* **15**, 670–5.
2. Vidugiriene, J. *et al.* (2014) Bioluminescent cell-based NAD(P)/NAD(P)H assays for rapid dinucleotide measurement and inhibitor screening. *Assay Drug Dev.Technol.* **12**, 514–26.
3. Leippe, D. *et al.* (2017) Bioluminescent assays for glucose and glutamine metabolism: High-throughput screening for changes in extracellular and intracellular metabolites. *SLAS Discov.* **22**, 366–77.

5.D. Related Products

Energy Metabolism Assays

Product	Size	Cat #
BCAA-Glo™ Assay	5ml	JE9300
Cholesterol/Cholesterol Ester-Glo™ Assay	5ml	J3190
Dehydrogenase-Glo™ Detection System	5ml	J9010
Glucose-Glo™ Assay	5ml	J6021
Glucose Uptake-Glo™ Assay	5ml	J1341
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
Glycerol-Glo™ Assay	5ml	J3150
Glycogen-Glo™ Assay	5ml	J5051
Lactate-Glo™ Assay	5ml	J5021
Malate-Glo™ Assay	5ml	JE9100
Metabolite-Glo™ Detection System	5ml	J9030
Triglyceride-Glo™ Assay	5ml	J3160

Additional sizes available.

Oxidative Stress Assays

Product	Size	Cat.#
GSH/GSSG-Glo™ Assay	10ml	V6611
NAD/NADH-Glo™ Assay	10ml	G9071
NADP/NADPH-Glo™ Assay	10ml	G9081
ROS-Glo™ H_2O_2 Assay	10ml	G8820

Additional sizes available.

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5.D. Related Products (continued)

Cell Viability, Cytotoxicity and Apoptosis Assays

Product	Size	Cat.#
Caspase-Glo® 3/7 Assay System	2.5ml	G8090
CellTiter-Glo® 2.0 Cell Viability Assay	10ml	G9241
CellTiter-Glo® 3D Cell Viability Assay	10ml	G9681
CellTiter-Fluor™ Cell Viability Assay	10ml	G6080
LDH-Glo™ Cytotoxicity Assay	10ml	J2380
RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay	100 assays	JA1011
RealTime-Glo™ MT Cell Viability Assay	100 assays	G9711

Additional sizes available.

6. Summary of Changes

The following changes were made to the 1/24 revision of this document:

1. In the legend for Figure 7, 2µM BHB standard was changed to 4µM BHB standard. Also, in this figure legend, 6µM HCl was changed to 6N HCl.

[a]U.S. Pat. Nos. 9,273,343 and 9,951,372, European Pat. No. 2751089, and Japanese Pat. No. 6067019.

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