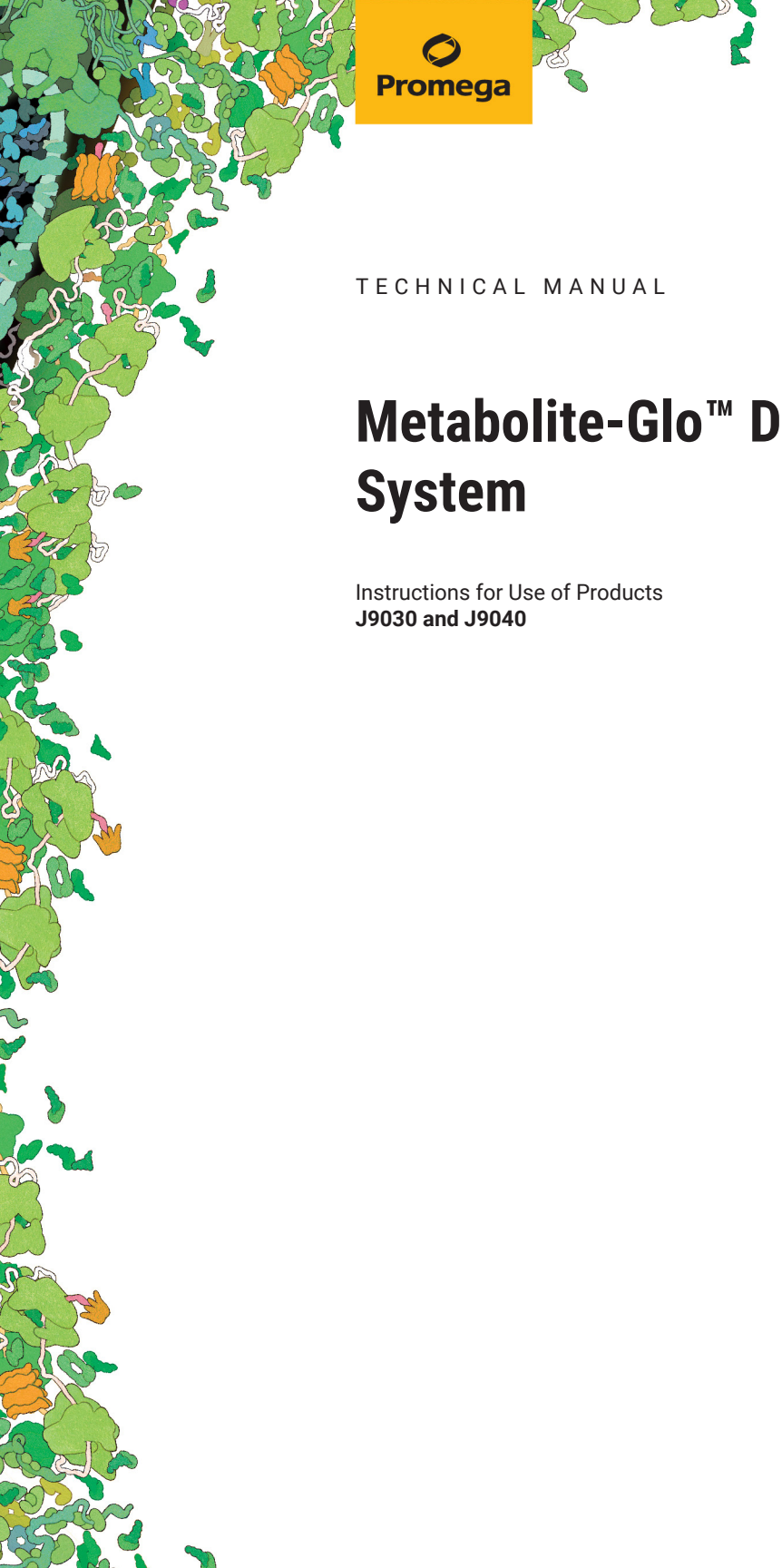

TECHNICAL MANUAL

Metabolite-Glo™ Detection System

Instructions for Use of Products
J9030 and J9040

11/23
TM715

Metabolite-Glo™ Detection System

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 Metabolite-Glo™ Detection System is based on the bioluminescent NAD(P)H detection technology. This kit can be used to build your own bioluminescence metabolite detection assays, which should be designed and optimized following the recommendations provided in this technical manual.

The Metabolite-Glo™ Detection System couples metabolite oxidation and NAD(P)H production with bioluminescent NAD(P)H detection (Figure 1; 1–3). To measure a metabolite of interest using this system, a metabolite-specific dehydrogenase must be identified. The selected dehydrogenase is then used to prepare a metabolite-specific detection reagent. The dehydrogenase couples metabolite oxidation with NAD(P)$^+$ reduction and reduced dinucleotides, NAD(P)H, are measured using a luciferase reaction. The amount of light produced by luciferase is proportional to the amount of metabolite in the sample. The NAD(P)H core technology does not discriminate between NADH and NAD(P)H (Figure 2), allowing either NAD-dependent or NADP-dependent dehydrogenases to be used for metabolite detection. The sensitivity and linearity of metabolite detection is defined by the core of NAD(P)H detection technology and is within 40nM–25µM range (Figure 2, Table 1). The oxidized forms, NAD$^+$ and NADP$^+$, are not detected and do not interfere with quantitation.

The Metabolite-Glo™ Detection System provides only the reagents required for bioluminescence NAD(P)H detection. To build your own metabolite assays, a metabolite-specific NAD- or NADP-dependent dehydrogenase needs to be identified and sourced by the user.

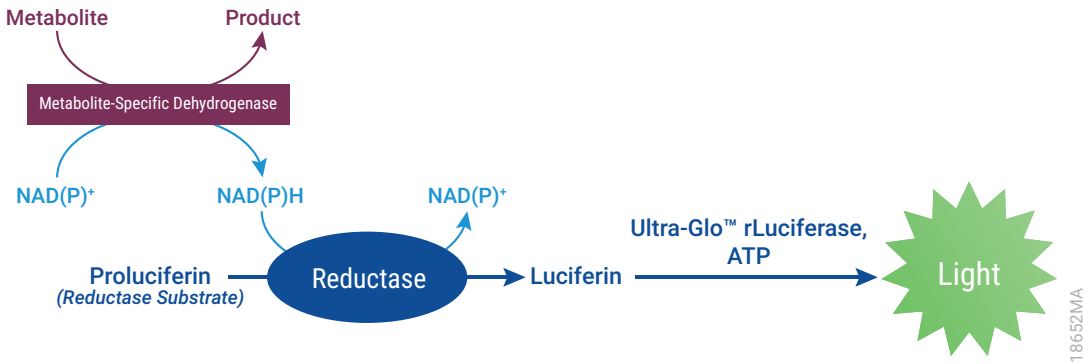

Figure 1. Schematic diagram of the Metabolite-Glo™ Detection System principle. A metabolite-specific dehydrogenase (not provided in this kit) catalyzes the oxidation of the metabolite with concomitant reduction of NAD$^+$ to NADH or NADP$^+$ to NADPH. In the presence of NADH or NADPH, Reductase enzymatically 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 metabolite in the sample.

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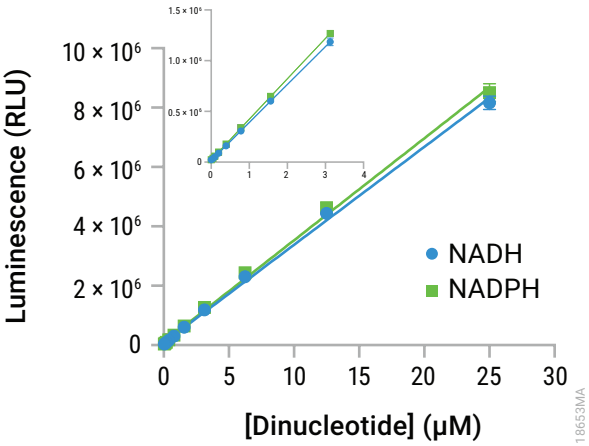

Figure 2. NADH/NADPH titration curves. NADH (Sigma Cat.# N6660) or NADPH (Sigma Cat.# N9910) were freshly prepared in phosphate-buffered saline (PBS) to 200µM. Twofold serial dilutions in the range of 25µM to 40nM were prepared in PBS. The negative control was PBS containing no NADH or NADPH. Aliquots of the freshly prepared dilutions (50µl) were transferred to a 96-well plate and metabolite detection reagent, without dehydrogenase and NAD/NADP, was added at a 1:1 ratio. Luminescence was measured in relative light units (RLU) using a GloMax® Discover (Cat.# GM3000). Metabolite detection reagent does not distinguish between NADH and NADPH. Each data point represents the average of four replicates. Error bars indicate ± one standard deviation. NADH data used to generate this figure are shown in Table 1.

Table 1. NADH Titration Data. Signal-to-background ratio (S/B) was calculated by dividing mean luminescence of samples by the mean luminescence of the negative control (no NADH). 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.

NADH (µM)	0	0.04	0.09	0.19	0.39	0.78	1.56	3.12	6.25	12.5	25
Average Luminescence (RLU × 10^3)	23	36	52	88	161	306	602	1,185	2,307	4,436	8,162
Standard Deviation (RLU × 10^3)	1.6	1.4	1.4	2.4	3.4	7.7	14.7	37.3	80.9	141	229
Coefficient of Variation (%)	7	4	3	3	2	3	2	3	4	3	3
S/B	1.0	1.6	2.3	3.9	7.1	13.6	26.7	52.6	102	197	362
S/N		7.9	18.3	40.2	85	174	355	712	1,399	2,702	4,985

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Metabolite-Glo™ Detection System	**5ml**	**J9030**

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

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

PRODUCT	SIZE	CAT.#
Metabolite-Glo™ Detection System	**50ml**	**J9040**

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

- 50ml Luciferin Detection Solution
- 275µl Reductase
- 275µl Reductase Substrate
- 1ml NAD
- 500µl NADP
- 15ml 0.6N HCl
- 15ml Neutralization Buffer

Storage Conditions: Store the Metabolite-Glo™ Detection System 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.

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. Considerations for Building Do-It-Yourself (DIY) Metabolite Assays

The Metabolite-Glo™ Detection System provides reagents required for bioluminescence NAD(P)H detection.

To build your own metabolite detection assay you will need to:

1. Identify a NAD- or NADP-dependent dehydrogenase that is specific for your metabolite of interest. If available, we recommend testing multiple dehydrogenase sources.
2. Optimize the amount of metabolite-specific dehydrogenase and NAD (for NAD-dependent dehydrogenase) or NADP (for NADP-dependent dehydrogenase) cofactors in the metabolite detection reagent.

   The optimal amount of dehydrogenase and NAD or NADP cofactors will vary, depending on the specific activity of the enzyme. We suggest starting with 200µM of NAD (40mM) or NADP (20mM), provided with this kit, and testing dehydrogenases within a broad range of activity (0.1–40U/ml). To ensure assay robustness, we recommend using a dehydrogenase concentration that is in excess, as shown in Figure 3, Panel A. In certain cases, if purified dehydrogenases are not readily accessible, lower enzyme concentrations (<0.1U/ml) may be sufficient. In addition, increasing NAD or NADP concentrations (400–800µM) might improve assay sensitivity (see example in Section 4.D).
3. Establish assay sensitivity and linearity by performing metabolite titration curves.

   For measuring metabolites in complex biological samples (e.g., cell lysates, tissue homogenates, serum, etc.) we recommend treating the samples with 0.6N HCl, which is provided in the kit, to inhibit endogenous dehydrogenase activity and hydrolyze reduced dinucleotides. To verify that your metabolite of interest is compatible with this treatment, prepare metabolite titration curves using the example protocol provided in Section 4.E.

4. Assay Optimization for Metabolite of Interest

Materials to Be Supplied By the User

- phosphate-buffered saline (PBS; e.g., Sigma Cat.# D8537 or GIBCO™ Cat.# 14190)
- 96-well assay plates (white with white or clear bottom; e.g., Corning® Cat.# 3903 or 3912)
- luminometer (e.g., GloMax® Discover System Cat.# GM3000)
- metabolite-specific dehydrogenase
- metabolite standard
- NADH or NADPH (Sigma Cat.# N6660, N9910)

4.A. Reagent Preparation

We recommend performing optimization reactions with 50µl of sample and 50µl of detection reagent in a 96-well plate. Alternative volumes can be used provided the 1:1 ratio of metabolite detection reagent volume to sample volume is maintained (e.g., 12.5µl of sample and 12.5µl metabolite detection reagent in a 384-well plate format).

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 metabolite detection reagent required. You will need 50µl for each assay in a 96-well plate with 50µl of sample. We recommend preparing additional reagent to compensate for pipetting errors.
3. Prepare metabolite detection reagent by combining components as shown in the table below.

   Note: Prepare only the volume of metabolite detection reagent calculated in Step 2. Unused metabolite 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, 40mM*	0.25µl	25µl
NADP, 20mM*	0.5µl	50µl
Dehydrogenase**	1µl	100µl

*Depending on dehydrogenase requirements, use NAD or NADP.

**Dehydrogenase stock concentration will vary. Assay conditions should be optimized as shown in Section 4.B.

4. Mix by gently inverting five times.

4.B. Assay Optimization Protocol

We recommend optimizing the assay using one metabolite concentration (e.g., 10µM) and testing variable dehydrogenase concentrations within the broad range of activity (0.1–40U/ml, final concentration in the detection reagent). We also recommend including NADH for NAD-dependent dehydrogenases or NADPH for NADP-dependent dehydrogenases.

1. Prepare a 10µM metabolite standard and 10µM NADH or 10µM NADPH control in PBS.

   Note: Metabolite and reduced dinucleotide (NADH/NADPH) standards are not included in this kit. NADH and NADPH are unstable and must be prepared immediately before use.
2. Transfer 50µl of metabolite and reduced dinucleotide (NADH or NADPH) standard to a 96-well plate. Include a no-metabolite (PBS only) negative control.

3. Prepare metabolite detection reagents as described in Section 4.A.

   Note: For initial testing we recommend preparing five different detection reagents and testing with controls using the guidelines shown in the following table.

	Detection Reagents to Prepare		
Component	**1–3 Dehydrogenase Concentrations**	**4 No Dehydrogenase Control**	**5 NADH/NADPH Control**
Dehydrogenase (DH)	+[1]	–	–
NAD or NADP	+	+	–
	Standard/Controls to Test		
10µM Metabolite Standard	yes	yes	not required
No-metabolite (PBS only) negative control	yes	yes	not required
10µM NADH/NADPH control	not required	not required	yes

[1]Prepare three dehydrogenase concentrations.

4. Add 50µl of detection reagents 1–4 to the wells containing metabolite standards and to the no-metabolite (PBS only) negative control.
5. Add 50µl of detection reagent 5 without dehydrogenase and NAD(P) to reduced dinucleotide (NADH or NADPH) control.
6. Mix by shaking the plate for 30–60 seconds.
7. Incubate at room temperature for 30–90 minutes.
8. Record luminescence every 30 minutes using a plate-reading luminometer, following the instrument manufacturer's instructions.

4.C. Example of Assay Optimization for Isocitrate Detection (NADP-Dependent)

Optimization assays were performed for isocitrate detection using the Metabolite-Glo™ Detection System and isocitrate dehydrogenase. Assay optimizations were performed using guidelines from Section 3, following the optimization protocol above.

Isocitrate dehydrogenase is a NADP-dependent enzyme that requires isocitrate and NADP to convert isocitrate to α-ketoglutarate. When coupled with the Metabolite-Glo™ Detection System, isocitrate dehydrogenase can be used to determine the concentration of D-isocitrate in samples.

Identifying the Optimal Isocitrate Dehydrogenase Concentration

As shown in Figure 3, Panel A, the isocitrate detection reagents prepared at different isocitrate dehydrogenase (Cayman Chemicals Cat.# 14131) concentrations had no effect on assay background (no-metabolite, PBS only) and showed more than a 125-fold increase in signal in the presence of 10µM D-isocitrate (Sigma Cat.# 58790). Only a slight increase in signal was observed when the isocitrate dehydrogenase concentration was increased from 0.001 to 0.004 U/ml with no further increase at 0.016 U/ml, indicating the isocitrate dehydrogenase is in excess. In addition, the light output generated with 10µM isocitrate corresponded to the light output generated with the 10µM NADPH control, consistent with complete conversion of isocitrate to NADPH.

Determining Incubation Time for Isocitrate Detection (Signal Plateau)

The oxidation of isocitrate is time dependent. Figure 3, Panel B, demonstrates that an incubation time of longer than 40 minutes at room temperature is required to achieve complete conversion. When completely converted, luminescence signal plateaus and remains stable for at least 30 minutes before starting to decrease. For consistent day-to-day results, we recommend determining the incubation time where the signal plateaus.

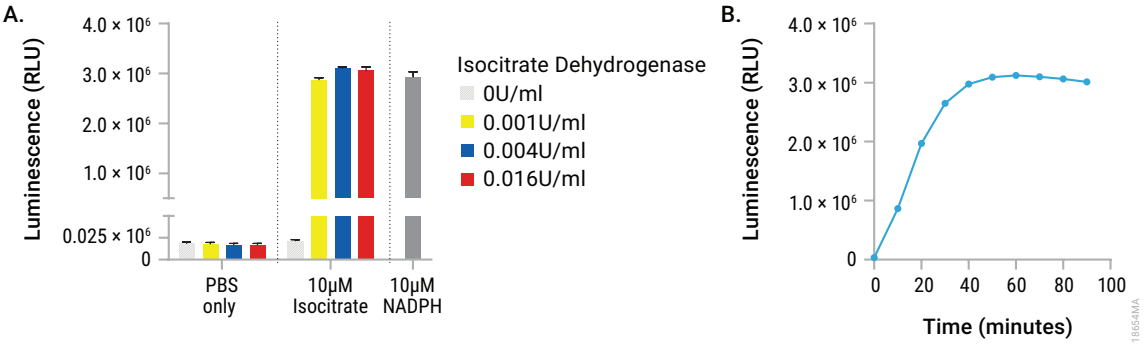

Figure 3. Optimization of isocitrate detection using the Metabolite-Glo™ Detection System. D-Isocitrate (Sigma, Cat.# 58790) was prepared as a 10mM stock in water. NADPH (Sigma Cat.# N6660) was prepared as 200µM stock in PBS before the experiment. For optimization, D-isocitrate and NADPH stocks were diluted in PBS to 10µM final concentration and 50µl was transferred into 96-well assay plate. Wells containing only PBS were included as negative controls. Isocitrate dehydrogenase (Cayman Chemicals Cat.# 14131) stock was prepared to 0.54U/ml in 50mM Tris (pH 7.5), 200mM NaCl and 20% glycerol from the provided solution and was stored at ≤65°C. To test isocitrate detection reagents containing different amounts of dehydrogenase, isocitrate dehydrogenase was diluted to 1.6, 0.4 and 0.1U/ml and added to the detection reagent to 0.016, 0.004 and 0.001U/ml final concentration. Detection reagent without isocitrate dehydrogenase was included as an assay background control. Prepared isocitrate detection reagents (50µl) were added to the samples and luminescence was read every 10 minutes for 90 minutes. After 60 minutes, signal from 10µM isocitrate detection with the three different isocitrate dehydrogenase concentrations was assessed in comparison to 10µM NADPH (**Panel A**). Over 90 minutes, a time-dependent increase in luminescence signal followed by a plateau in signal with detection reagent at 0.004U/ml of isocitrate dehydrogenase was demonstrated (**Panel B**). Data represent the average of 3–4 replicates and error bars indicate ± one standard deviation.

4.C. Example of Assay Optimization for Isocitrate Detection (NADP-Dependent; continued)

Optimal Assay Conditions for Isocitrate Detection

Based on the overall data, optimal conditions for isocitrate detection include isocitrate detection reagent made with 0.004–0.016U/ml of isocitrate dehydrogenase and 200µM NADP, with a 60-minute room temperature incubation time. A full titration curve was performed with optimal assay conditions following the protocol in Section 4.E and used to determine assay linearity and sensitivity.

4.D. Example of Assay Optimization for D-2-Hydroxyglutarate Detection (NAD-Dependent)

D-2-hydroxyglutarate (D2HG) is primarily used as a biomarker for metabolic disease or various cancers associated with isocitrate dehydrogenase mutations (4,5). For detection of D2HG, D2HG dehydrogenase was selected and is an NAD-dependent enzyme that requires D2HG and NAD to convert D2HG to 2-oxoglutarate. When coupled with the Metabolite-Glo™ Detection System, D2HG dehydrogenase can be used to determine the D2HG concentration in samples.

Determining D2HG Dehydrogenase Concentration

In contrast to the example described in Section 4.C, an initial D2GH dehydrogenase screen was set up at 0.04, 0.02 and 0.01U/ml, a narrower range of enzyme activity. As shown in Figure 4, Panel A, the presence of D2GH dehydrogenase in the detection reagent did not increase the assay background and D2HG specific signal was measured at all dehydrogenase concentrations. Luminescence signal increased with increasing dehydrogenase concentrations. However, contrary to the example in Section 4.C, the maximum signal with 10µM D2HG (Selleck Chem Cat.# S7873) was lower in comparison to the signal produced from 10µM NADH, indicating an incomplete conversion of D2HG to NADH. Furthermore, there is no signal plateau for any of the D2HG dehydrogenase concentrations, even after 90 minutes, indicating there is still D2HG to be detected.

Increasing NAD Concentration for D2HG Detection

Initial testing, which varied the D2HG dehydrogenase concentration in metabolite detection reagent, did not produce optimal assay conditions. Another variable that may improve D2HG detection in the assay is increasing NAD concentration. As depicted in Figure 4, Panel B, increasing NAD concentration from 200µM to 800µM increased signal output and reaction rate, as shown by D2HG detection over time. Furthermore, a plateau in the signal is observed at 60–90 minutes with 800µM NAD in D2HG detection reagent, demonstrating that this is an appropriate incubation time for D2HG detection.

Optimized Assay Conditions for D2HG Detection

Based on these results, the optimal conditions for D2HG detection are 0.02U/ml of D2HG dehydrogenase and 800µM NAD in D2HG detection reagent, with a 60-minute room temperature incubation. As shown in Figure 4, Panel C, under these optimal conditions, 200nM of D2HG was measured with twofold signal above the assay background and the assay was linear up to 25µM of D2HG.

This example illustrates that, although complete metabolite conversion and the use of excess dehydrogenase is preferred, when the dehydrogenase supply is limited, the assay can be set up under suboptimal conditions. For standard metabolite detection conditions, we recommend using 200µM of required dinucleotide (NAD or NADP). However, as shown in this example, for some dehydrogenases higher dinucleotide concentrations might increase assay sensitivity.

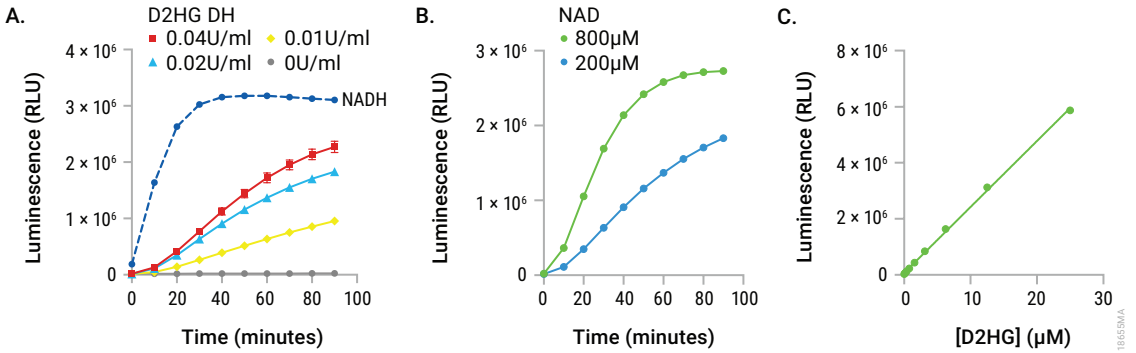

Figure 4. Optimizing D2HG detection using the Metabolite-Glo™ Detection System. D-2-hydroxyglutarate (D2HG) and newly reconstituted NADH were diluted to 10µM in PBS and 50µl was added to a 96-well plate. D2HG detection reagents containing 200µM NAD and different amounts (0.04, 0.02, 0.01U/ml) of D2HG dehydrogenase (Sigma Cat.# SAE0097) were prepared as described in Section 4.A and added to the wells containing 10µM D2HG. For NADH control, detection reagent without NAD or dehydrogenase was prepared and added to the wells containing 10µM NADH. The reagents were added at 1:1 ratio and luminescence was read every 10 minutes for 90 minutes (**Panel A**). D2HG detection reagent was prepared containing 0.02U/ml D2HG dehydrogenase and 200µM or 800µM NAD. Prepared D2HG detection reagents (50µl) were added to 50µl of 10µM D2HG samples in PBS and luminescence was read every 10 minutes for 90 minutes (**Panel B)**. A D2HG titration curve was then performed with optimized assay conditions, including D2HG detection reagent with 0.02U/ml D2HG dehydrogenase and 800µM NAD, with a 60-minute room temperature incubation (**Panel C**). Data represent the average of 3–4 replicates and error bars indicate ± one standard deviation.

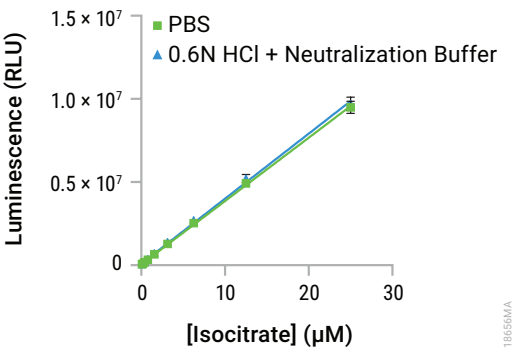

Figure 5. Compatibility of isocitrate detection with acid treatment. Isocitrate was titrated twofold from 50µM in PBS. Isocitrate detection was performed as described in the above example protocol. Samples treated with 0.6N HCl followed by Neutralization Buffer or with PBS only show overlapping curves, indicating the isocitrate detection assay is compatible with acid treatment. Data represents the average of four replicates and error bars indicate ± one standard deviation.

4.E. Example Protocol for Generating Metabolite Titration Curves

After initial assay optimization, determine assay sensitivity and linearity by performing a metabolite titration curve. For measuring metabolites in complex biological samples, we recommend using 0.6N HCl (acid) and Neutralization Buffer (1M Tris base) for sample preparation. Acid treatment rapidly stops metabolism, inhibits endogenous protein activity and destroys reduced NAD(P)H dinucleotides.

Therefore, to establish assay sensitivity and linearity, we recommend comparing the metabolite detection assay with and without 0.6N HCl and Neutralization Buffer, using PBS instead of treatment, for comparison. Here we provide an example protocol using isocitrate.

1. Titrate D-isocitrate from 50µM in PBS and add 25µl to a 96-well plate. Include a no-isocitrate control (PBS only).
2. Add 12.5µl of 0.6N HCl (included in this kit) or PBS to each well, shake the plate for 30–60 seconds to mix, and incubate for 5 minutes at room temperature.
3. Add 12.5µl of Neutralization Buffer (included in this kit) or PBS, shake for 30–60 seconds to mix.

   Note: Adding acid and neutralizing dilutes the sample twofold, remaining within the linear range of this assay (25µM).
4. Prepare isocitrate detection reagent, optimized in Section 4.C, as follows:

Component	Volume Per Reaction	Volume Per 100 Reactions
Luciferin Detection Solution	50µl	5ml
Reductase Substrate	0.25µl	25µl
Reductase	0.25µl	25µl
NADP	0.50µl	50µl
Isocitrate dehydrogenase (0.54U/ml)	0.40µl	40µl

   Note: Isocitrate was diluted from the provided stock solution to 0.54U/ml in 50mM Tris(pH7.5) with 200mM NaCl and 20% glycerol.
5. Add 50µl of isocitrate detection reagent to each well.
6. Mix by shaking the plate for 30–60 seconds.
7. Incubate at room temperature for 60 minutes.
8. Record luminescence using a plate-reading luminometer, following the instrument manufacturer's instructions.

5. Assay Validation in a Biological System

Assay validation in a relevant biological system is recommended to ensure the optimized metabolite-specific assay performs as intended. This includes understanding the background of the system and if additional sample preparations are required. For most biological samples (e.g., cell supernatants, cell lysates, serum and tissue homogenates), we strongly recommend using the 0.6N HCl and Neutralization Buffer supplied with the kit. Prior to performing experiments using acid/base treatment, ensure that the treatment is compatible with the metabolite of interest, as shown in Section 4.E. Acid-treated and neutralized samples can be assayed immediately or stored at –20°C before assay.

To illustrate the use of the assay for measuring metabolites in biological samples, Figure 6 shows isocitrate detection in mammalian cell lysates. The data show a proportional increase in signal with increasing cell numbers, suggesting that isocitrate can be successfully measured in biological samples using the core bioluminescence NAD(P)H detection technology after optimizing for a specific metabolite detection.

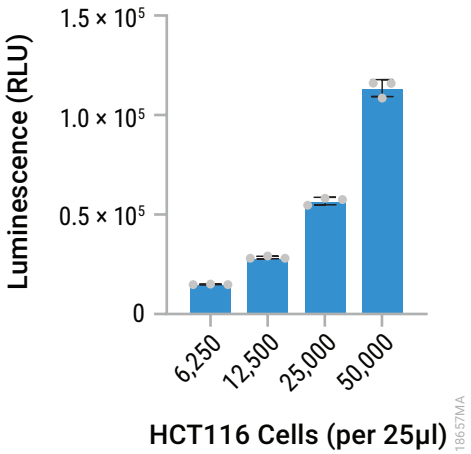

Figure 6. Intracellular isocitrate detection. HCT116 cells were collected, washed with cold PBS and diluted twofold from 50,000 to 6,250 cells in 25µl. Isocitrate was measured as described in the example protocol in Section 4.E. Cells (25µl) were transferred into a 96-well assay plate. Wells with no cells (PBS only) were included for assay background control. 0.6N HCl (12.5µl) was added to all samples for cell lysis, enzyme inactivation and elimination of reduced dinucleotides. The samples were then neutralized with 12.5µl Neutralization Buffer. Isocitrate detection reagent (50µl) was prepared as described in Section 4.E, added to samples and, after a 60-minute incubation at room temperature, luminescence was read. The average luminescence for three replicates (gray dots) without background (no cells, PBS only) is shown in relative light units (RLU).

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6. Appendix

6.A. Dehydrogenase Activity in Metabolite Detection Reagent

The Metabolite-Glo™ Detection System couples two reactions that, in most cases, can be run simultaneously. The first reaction uses a metabolite-specific dehydrogenase with the required dinucleotide (NAD or NADP) to oxidize the metabolite of interest, while simultaneously reducing NAD or NADP to NAD(P)H. The second reaction uses the reduced dinucleotides to convert proluciferin to luciferin, which creates light (in the presence of luciferase and ATP) that is proportional to the concentration of the metabolite. Most of the dehydrogenases tested are compatible with metabolite detection reagents. However, if a metabolite-specific dehydrogenase compatible with metabolite detection reagent cannot be identified, using a two-step reaction can be considered. To run a two-step reaction, first add NAD(P) and metabolite-specific dehydrogenase to the sample in a buffer compatible with enzymatic activity. Second, detect the NAD(P)H formed in the first reaction by adding an equal volume of metabolite detection reagent without NAD(P) and dehydrogenase.

6.B. Additional Sample Preparation Considerations

Cell supernatants, cell lysates, serum, tissue homogenates and many other biological sample types have been successfully tested with the Metabolite-Glo™ Detection System. Before collecting data, each sample type should be validated for compatibility following the guidance provided in Section 5.

For most samples, treating with 0.6N HCl will inactivate endogenous enzymes and lyse cells (if applicable). When dealing with difficult-to-lyse samples such as 3D cultures, Triton® X-100 can be added with acid treatment, after first testing compatibility. We do not recommend using detergent lysis without acid treatment, since many dehydrogenases remain active in detergent-lysed samples, significantly increasing the assay background. 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 metabolite and metabolite-specific dehydrogenase.

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

6.C. 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.

6.D. 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 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, 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-half area (Corning® Cat.# 3696), 384-well (Costar® Cat.# 3570) or 384-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.

6.E. References

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4. Dang, L. *et al.* (2009) Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. *Nature* **462**, 739–44.
5. Leonardi, R. *et al.* (2012) Cancer-associated isocitrate dehydrogenase mutations inactivate NADPH-dependent reductive carboxylation. *J. Biol. Chem.* **287**, 14615–20.

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6.F. Related Products

Energy Metabolism Assays

Product	Size	Cat.#
Glucose Uptake-Glo™ Assay	5ml	J1341
Lactate-Glo™ Assay	5ml	J5021
Malate-Glo™ Assay	5ml	JE9100
Pyruvate-Glo™ Assay	5ml	J4051
Glucose-Glo™ Assay	5ml	J6021
Glycogen-Glo™ Assay	5ml	J5051
Glutamine/Glutamate-Glo™ Assay	5ml	J8021
BHB-Glo™ (Ketone Body) Assay	5ml	JE9500
Glycerol-Glo™ Assay	5ml	J3150
Triglyceride-Glo™ Assay	5ml	J3160
Cholesterol/Cholesterol Ester-Glo™ Assay	5ml	J3190
BCAA-Glo™ Assay	5ml	JE9300
Dehydrogenase-Glo™ Detection System	5ml	J9010

Additional sizes available.

Oxidative Stress Assays

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

Additional sizes available.

Cell Viability, Cytotoxicity and Apoptosis Assays

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

Additional sizes available.

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