

REPORTER SYSTEMS

BioLux[®]
Gaussia Luciferase Assay Kit

Instruction Manual

NEB #E3300S/L
100/1,000 assays
Version 2.0 11/14



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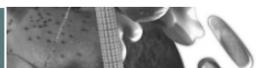


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Kit Includes:

	#E3300S	#E3300L
BioLux GLuc Assay Buffer (1X)	5 ml.....	2 x 25 ml
BioLux GLuc Stabilizer (100X)	0.800 ml.....	.8 ml
BioLux GLuc Substrate (100X).....	0.05 ml.....	0.500 ml

(Kit components are not sold separately)

Storage Information:

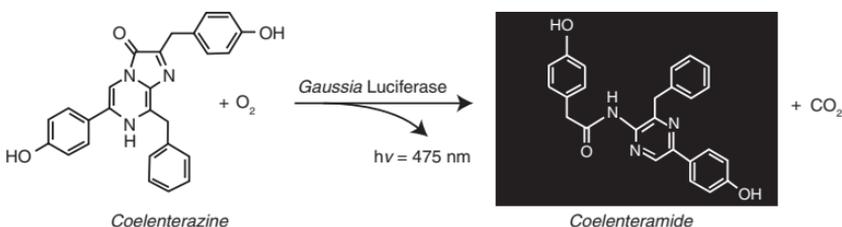
BioLux *Gaussia* Luciferase Assay Buffer and Stabilizer can be stored at 4°C.

BioLux *Gaussia* Luciferase Substrate must be tightly capped and stored at -20°C.

Method Overview:

The BioLux *Gaussia* Luciferase Assay Kit contains the reagents necessary for assaying *Gaussia* Luciferase (GLuc) activity, most commonly from cell culture supernatants. *Gaussia* Luciferase is a luciferase protein from the marine copepod *Gaussia princeps* (1,2). *Gaussia* luciferase can be expressed in mammalian cells using reporter plasmids available from NEB (refer to the Companion Products). This luciferase, which does not require ATP, catalyzes the oxidation of the substrate coelenterazine in a reaction that produces light (Figure 1), and has considerable advantages over other luminescent reporter genes.

Figure 1: The Photo-oxidation catalyzed by *Gaussia* Luciferase.



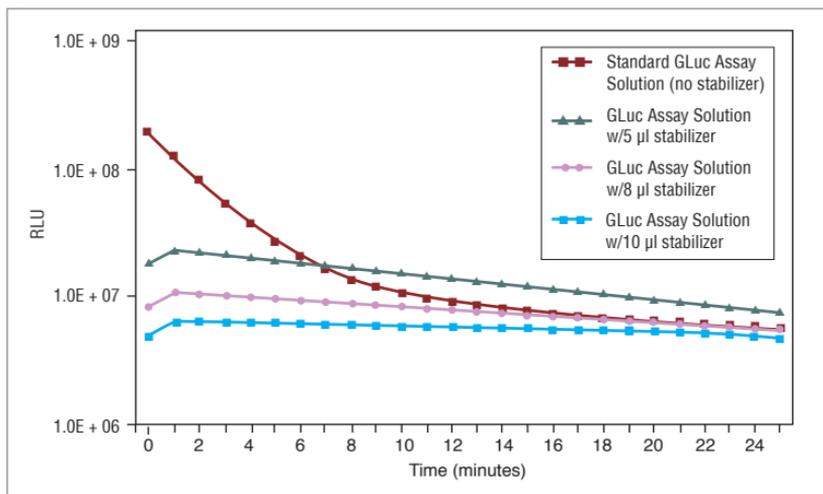
Haddock, S.H.D., McDougall, C.M. and Case, J.F., *The Bioluminescence Web Page*, <http://lifesci.ucsb.edu/~biolum/> (created 1997; updated 2005).

This kit now includes an additional stabilizer component, which allows the use of the assay in high throughput format or without the requirement of an injector-equipped luminometer. This three-component assay system provides the user with 2 options: (a) use the assay without stabilizer for enhanced light output or (b) use with the desired amount of stabilizer for enhanced signal stability. The stabilizer component allows the use of the assay in high throughput format or without the requirement of an injector-luminometer.

For standard assays giving the highest activity, the kit can be used with the GLuc substrate mixed in the assay buffer. With the stabilized assay protocols, the light emission decays slowly with a half-life of approximately 25 minutes. The addition of stabilizer decreases the absolute value of light output but confers signal stability over time (Figure 2).

The luminescence measured from the supernatant of cultured cells transfected with a plasmid expressing GLuc is proportional to the amount of enzyme produced, which in turn, reflects the level of transcription. Alternatively, a cell lysate can be used for the assay. Although most of the activity is secreted, the high sensitivity of GLuc allows measurements from the cellular fraction as well.

Figure 2: GLuc kinetics using the BioLux GLuc Assay Kit in either standard or stabilized assay.



Assays were setup using assay solution without stabilizer or with the indicated amounts of stabilizer (5 µl, 8 µl or 10 µl of stabilizer per 50 µl GLuc assay solution).

Advantages:

Gaussia Luciferase possesses a natural secretory signal and upon expression is secreted into the cell medium. Therefore, lysing cells in order to assay GLuc activity is not necessary. As a result, GLuc is an ideal reporter gene for time course studies (3).

Gaussia Luciferase generates over 1000-fold higher bioluminescent signal intensity when compared to Firefly or *Renilla* Luciferases, making it an ideal transcriptional reporter (3).

GLuc shows the highest reported activity of any characterized luciferases (4).

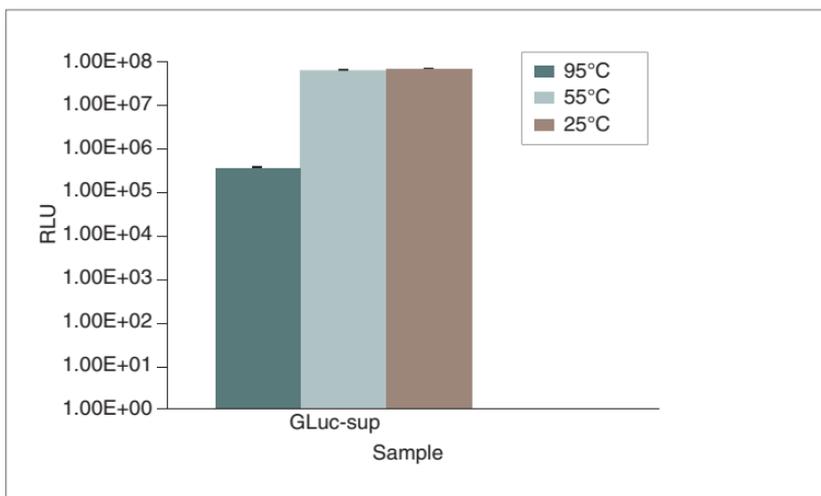
The secreted protein is thermally stable (Figure 3) and has extremely high activity in light production allowing for very sensitive assays.

The secreted GLuc is also very stable in the presence of 55 µM β-mercaptoethanol, which is typically used in culturing mouse stem cells (Figure 4).

The GLuc containing samples (i.e. growth media or cell lysates) can be stored at -20°C for long-term storage or at 4°C for several days without loss of activity.

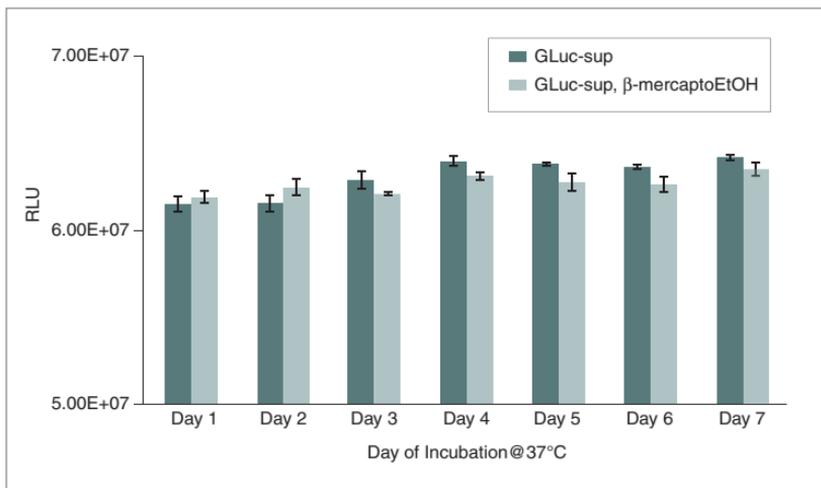
The stabilizer component of this assay system provides steady kinetics over a longer time period allowing users the time required for high-throughput analysis as well as manually delivered assays.

Figure 3: Stability of *Gaussia* Luciferase at various temperatures.



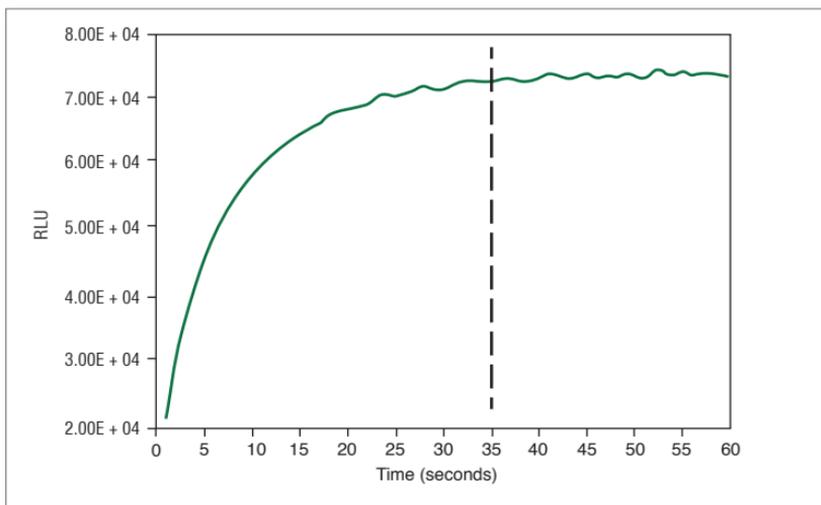
Growth media from *GLuc*-expressing cells (*GLuc*-sup) were incubated at 95°C and 55°C for 30 minutes and allowed to cool to room temperature (25°C) before assaying for *GLuc* activity.

Figure 4: Stability of *Gaussia* Luciferase at 37°C over a period of seven days.



Growth media from *GLuc*-expressing cells grown in $\pm\beta$ -mercaptoethanol-containing media (*GLuc*-sup & *GLuc*-sup, β -mercaptoEtOH) were placed at 37°C and assayed everyday for a period of seven days.

Figure 5: *Gussia* Luciferase activity after adding GLuc assay solution containing stabilizer to a sample.



The GLuc assay solution containing stabilizer (i.e. 8 μ l of stabilizer per 50 μ l GLuc assay solution) was added to a GLuc sample and the measurements were taken at 1-second increments (see Usage Notes).

BioLux *Gussia* Luciferase Assay Kit Protocols:

Standard Assay Protocol I (Luminometers without injectors):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 μ l of BioLux GLuc Substrate to 5 ml of BioLux GLuc Assay Buffer immediately before performing the assay.
2. Mix well by inverting the tube several times (Do not vortex).
3. Set the luminometer for 2–10 seconds of integration.
4. Pipet samples* (5–20 μ l per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Add the GLuc assay solution (50 μ l) to a sample (i.e. Add the assay solution to only one sample at a time) and promptly measure the luminescence.
6. Repeat Step 5 for all samples.

Standard Assay Protocol II (Injector-equipped luminometers):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 μ l of BioLux GLuc Substrate to 5 ml of BioLux GLuc Assay Buffer immediately before performing the assay. (Be sure to prepare enough assay solution as needed for all samples as well as for priming a particular luminometer as recommended by the manufacturer).

2. Mix well by inverting the tube several times (Do not vortex).
3. Set the luminometer with the following parameters: 50 μ l of injection volume and 2–10 seconds of signal integration.
4. Pipet samples* (5–20 μ l per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Prime the injector with the GLuc assay solution and proceed with the measurement.

Stabilized Assay Protocol I (Luminometers without injectors):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 μ l of BioLux GLuc Substrate and 800 μ l of BioLux GLuc Stabilizer to 5 ml of BioLux GLuc Assay Buffer.
2. Mix well by inverting the tube several times (Do not vortex).
3. Incubate at room temperature for 25 minutes (protect from light in a tightly capped tube/bottle) before adding to the sample.
4. Set the luminometer for 2–10 seconds of integration.
5. Pipet samples* (5–20 μ l per well) into a 96-well plate (opaque, white or black) or a tube.
6. Add the assay solution (50 μ l per well) to all samples.
7. Incubate at room temperature for 35–40 seconds (refer to Notes) and proceed with the measurement.

Stabilized Assay Protocol II (Injector-equipped luminometers):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 μ l of BioLux GLuc Substrate and 800 μ l of BioLux GLuc Stabilizer to 5 ml of BioLux GLuc Assay Buffer (Be sure to prepare enough assay solution as needed for all samples as well as for priming a particular luminometer as recommended by the manufacturer).
2. Mix well by inverting the tube several times (Do not vortex).
3. Incubate at room temperature for 25 minutes (protect from light in a tightly capped tube/bottle) before adding to the sample.
4. Set the luminometer with following parameters: 50 μ l of injection, 35–40 seconds of delay (refer to Usage Notes), & 2–10 seconds of integration.
5. Pipet samples* (5–20 μ l per well) into a 96-well plate (opaque, white or black) or a tube.

6. Prime the injector with the assay solution and proceed with the measurement.

* Approximately 90% of GLuc is secreted out into the growth media after transfection and thus, the GLuc activity is typically assayed from the supernatant (i.e. growth media of GLuc-transfected cells). However, as long as the cells are alive, approximately 10% of GLuc is present inside the cell. Therefore, Gluc activity can also be assayed from the cell lysate. We recommend that the cell lysates be prepared by using Luciferase Cell Lysis buffer (NEB #B3321), since this lysis buffer is designed to be compatible with Cypridina, Gaussia, Renilla, Firefly luciferase and β -galactosidase.

Usage Notes:

Because of the stability of GLuc, the activity measured in the growth media of a GLuc-expressing culture reflects the protein that has accumulated up to the time of sampling.

For the standard assay solution, i.e. solution that does not contain stabilizer, equilibration of the assay solution is not necessary. After adding the GLuc assay solution to the sample, we recommend a delay of 1–5 seconds before taking a measurement. Keeping the delay time consistent across experiments will ensure reproducibility.

For the stabilized assay solution, i.e., the stabilizer-containing GLuc assay solution, the solution should be equilibrated at room temperature for 25 minutes (protect from light in a tightly capped tube/bottle) before adding to the sample. After adding the equilibrated GLuc assay solution to the sample, we recommend a delay time of 35–40 seconds before taking a measurement in order to reach maximum level of detection. This is especially important when the GLuc activity level is low (e.g. $< e4$ RLU). For example, the readout obtained after 35–40 seconds of delay is $\sim e4$; when compared to 30, 20 and 10 seconds of delay, the readouts are as follows: $\sim 2\%$ decrease (for 30 seconds of delay), $\sim 7\%$ decrease (for 20 seconds of delay), & $\sim 20\%$ decrease (for 10 seconds of delay) (refer to Figure 5).

Use the prepared GLuc assay solution within 24 hours. The unused portion of the assay solution should be tightly capped and stored at -20°C . It should be completely thawed (in the dark) to room temperature before use.

The linear range of the luminometer used for the assay must be established. This is easily done by assaying serial dilutions of a sample. In addition, the assay solution itself as well as the conditioned media (i.e. growth media from untransfected cells) should be included to establish the background signal in the assay.

If excess activity for the instrument range is found, the sample should be diluted in PBS or 10% serum-containing media. The integration time can also be reduced (e.g. 2 seconds instead of 5 seconds).

When assaying the serial dilutions of a sample, it is best to assay the most diluted samples first & the most concentrated samples last. This will help to minimize false readings, i.e. cross talk effect in which signals from samples of high RLU cross into the next sample. The cross-talk effect seems to be more pronounced when plates (white or black) with clear-bottoms are used.

Frequently Asked Questions:

Can the BioLux GLuc Assay Kit be used for measuring Renilla luciferase activity?

Yes. The substrate, coelenterazine, is oxidized in the light reaction catalyzed by *Gaussia* or *Renilla* luciferase. Therefore, the GLuc assay reagents can be used for measuring *Renilla* luciferase activity. However, we recommend that the cell lysate be prepared by using the Luciferase Cell Lysis Buffer (NEB #B3321), since *Renilla* activity assay requires lysing the cells.

Can I assay Gaussia and Renilla luciferase activities if reporter genes are co-transfected in the cells?

No. *Gaussia* and *Renilla* catalyze the light reaction using the same substrate. Thus, the activities of these two luciferases can't be distinguished in the same cells expressing these reporter genes.

Can I assay Gaussia and Firefly luciferase activities if reporter genes are co-transfected in the cells?

Yes. *Gaussia* and Firefly luciferases catalyze the light reaction using different substrates. Therefore, the activity of each luciferase can easily be assayed from the same cells expressing both reporters. The GLuc and the Firefly luciferase activities do not cross-react with each other. The GLuc activity is typically assayed from the supernatant, but it also can be obtained from the cell lysate. The Firefly luciferase activity, on the other hand, can only be assayed from the cell lysate.

Can I add GLuc assay working solution directly to the cells?

Yes. You must establish that your instrument will provide readings within its linear range.

Is the BioLux Gaussia Luciferase Substrate stored at -20°C still good 3 months after the expiration date?

Yes. A 9-month old substrate can be expected to lose ~1/2 log in activity when compared to the freshly made substrate.

Can the BioLux Gaussia Luciferase Assay Kit be used to assay GLuc activity in GLuc-containing blood samples and GLuc samples containing high serum?

Yes. However, to analyze the data it is important to have the proper controls (GLuc assay solution alone; GLuc-containing blood sample alone; or GLuc sample containing high serum alone) in the assay, in order to establish the background signal. The presence of serum tends to increase the background signal of the GLuc activity measurements.

Can I use the BioLux Gaussia Luciferase Assay Kit to assay GLuc activity in an in vivo model, i.e., injecting the assay solution into a mouse?

No. This assay system is not designed for use in *in vivo* applications.

What is the concentration of coelenterazine in the kit?

The formulations of all kit components are proprietary.

Where can I find the NEB's kit cited as BioLux GLuc Flex Assay Kit, E3308?

The E3308 kits have been replaced by the current E3300, which now includes the stabilizer component. The Stabilized Assay Protocols (I & II) of the current E3300 are equivalent to those of the E3308.

References:

1. Verhaegen M. and Christopoulos T.K. (2002) *Anal. Chem.* 74, 4378–4385.
2. Tannous, B.A., Kim, D.E., Fernandez, J.L., Weissleder, R., and Breakefield, X.O. (2005) *Mol. Ther.* 11, 435–443.
3. Wu, C., Suzuki-Ogoh, C. and Ohmiya, Y. (2007) *BioTechniques* 42, 290–292.
4. Goerke, A., Loening, A., et al. (2008) *Metabolic Engineering.* 10,187–200.

Ordering Information

PRODUCT	NEB #	SIZE
BioLux <i>Gaussia</i> Luciferase Assay Kit	E3300S/L	100/1,000 assays
COMPANION PRODUCTS		
Anti-GLuc Antibody	E8023S	0.2 ml
pCMV-GLuc 2 Control Plasmid	N8081S	20 µg
pSV40-GLuc Control Plasmid	N0323S	20 µg
pGLuc-Basic 2 Vector	N8082S	20 µg
pTK-GLuc Vector	N8084S	20 µg
pGLuc Mini-TK 2 Vector	N8086S	20 µg
BioLux <i>Cypridina</i> Luciferase Assay Kit	E3309S/L	100/1,000 assays
pCMV-CLuc 2 Control Plasmid	N0321S	20 µg
pSV40-CLuc Control Plasmid	N0318S	20 µg
pCLuc-Basic 2 Vector	N0317S	20 µg
pTK-CLuc Vector	N0322S	20 µg
pCLuc Mini-TK 2 Vector	N0324S	20 µg



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