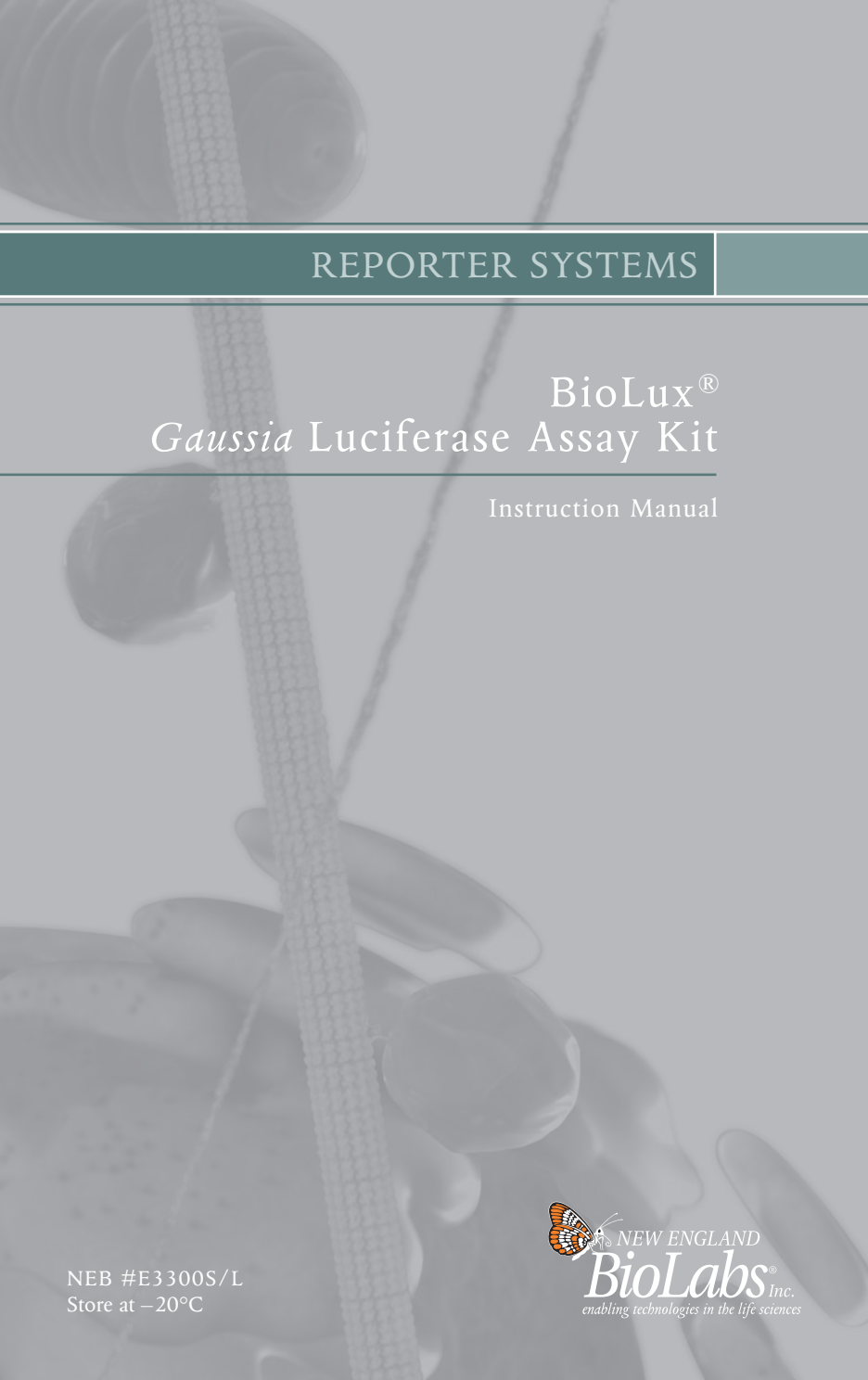


REPORTER SYSTEMS

BioLux®
Gaussia Luciferase Assay Kit

Instruction Manual

NEB #E3300S/L
Store at –20°C

NEW ENGLAND
BioLabs® Inc.
enabling technologies in the life sciences

Table of Contents:

Kit Includes:

	#E3300S	#E3300L
BioLux® GLuc Assay Buffer (1X)	5 ml	2 x 25 ml
BioLux® GLuc Substrate (100X)	0.05 ml	0.5 ml

(Kit components are not sold separately)

Storage Information:

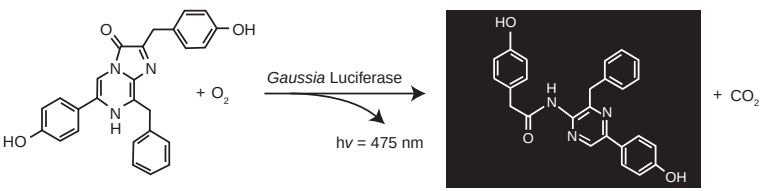
BioLux *Gaussia* Luciferase Assay Buffer can be stored at 4°C.

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 reporter luciferase 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 emits light (Figure 1), and has considerable advantages over other reporter genes.

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



Coelenterazine *Coelenteramide*

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

Advantages:

***Gaussia* Luciferase possesses a natural secretory signal and upon expression is secreted into the cell medium. Therefore, lysis of the cells is not necessary.**

***Gaussia* Luciferase generates over 1000-fold higher bioluminescent signal intensity when compared to Firefly or *Renilla* Luciferases, making it an ideal transcriptional reporter (2).**

The secreted protein is thermally stable (Figure 2) and has extremely high activity in light production allowing for very sensitive assays.

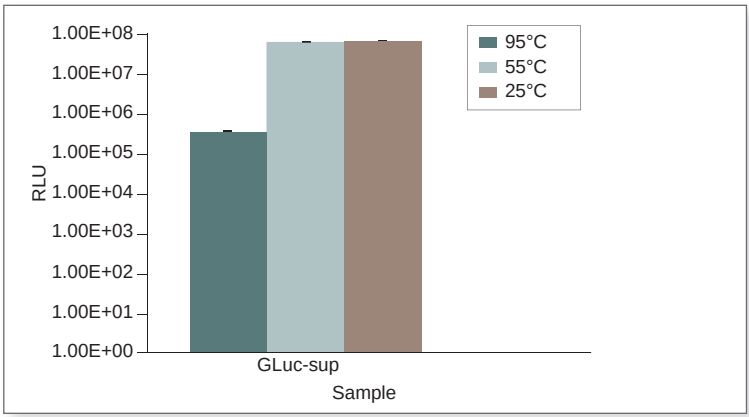
The secreted GLuc is also very stable in the presence of 55 µM β-mercaptoethanol, which is typically used in culturing mouse stem cells (Figure 3).

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 *Gaussia* Luciferase Assay Kit has been designed to stabilize light production with robust kinetics, which gives more consistent results (Figure 4).

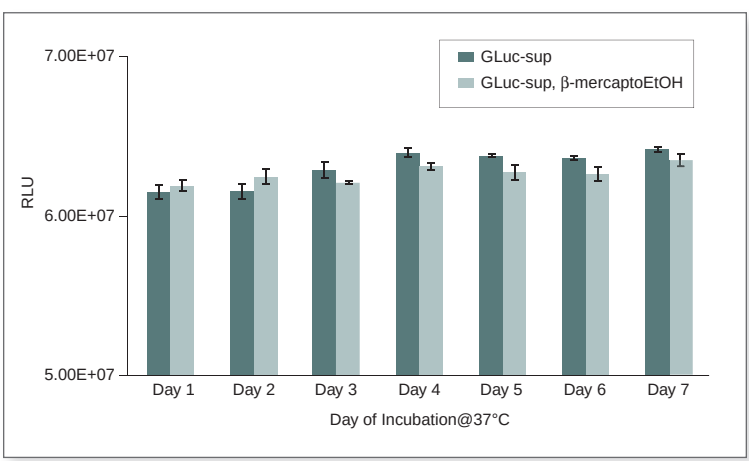
A simple three step process (Figure 5) makes it easy to assay multiple samples and collect data at different time points.

Figure 2: Stability of *Gaussia* Luciferase at various temperatures.



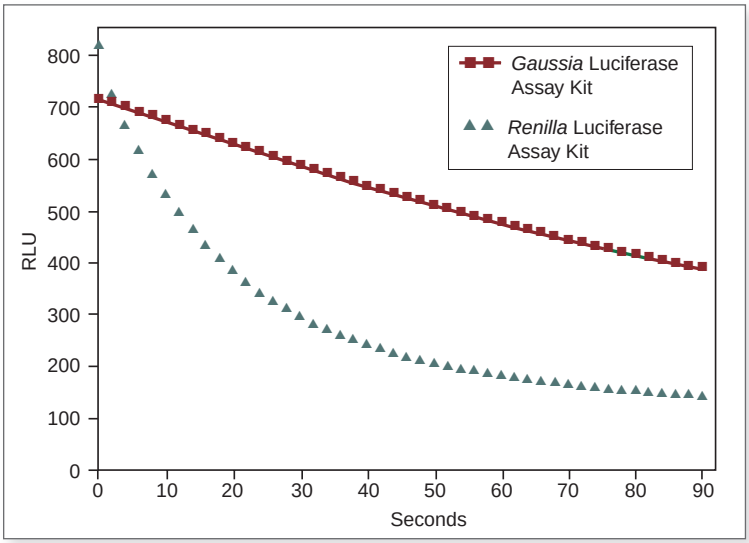
Growth media from GLuc-expressing cells (GLuc-sup) were incubated at 95°C and 55°C for 30 minutes and allowed to cool to room temperature (25°C) before assaying for GLuc activity.

Figure 3: Stability of *Gaussia* Luciferase at 37°C over a period of 7 days.



Growth media from GLuc-expressing cells grown in ±β-mercaptoethanol-containing media (GLuc-sup & GLuc-sup, β-mercaptoEtOH) were placed at 37°C and assayed everyday for a period of 7 days.

Figure 4: *Gaussia* & *Renilla* assay systems.



Light reaction of GLuc secreted from mammalian cells using the Gaussia Luciferase Assay Kit versus a commercially available Renilla Assay Kit.

BioLux *Gaussia* Luciferase Assay Kit Protocols:

Protocol I (Luminometers without injectors):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl 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 µl per well) into a 96-well white (opaque) or black plate, or a luminometer tube.
5. Add the GLuc assay solution (50 µl) 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.

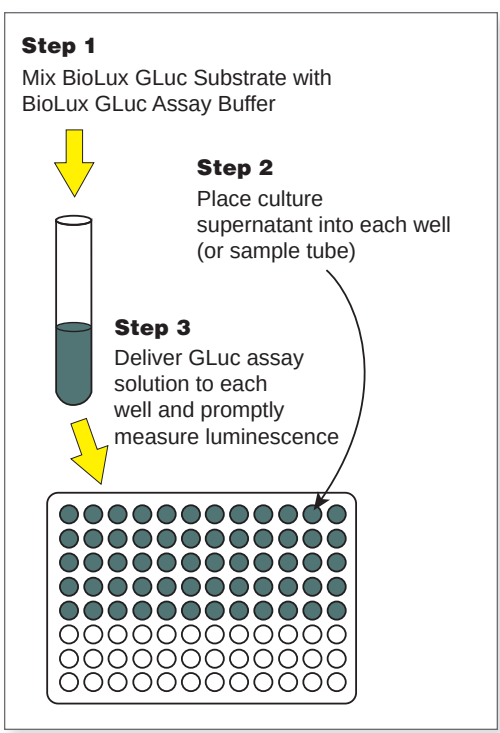
Protocol II (Injector-equipped luminometers):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl 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 µl of injection volume and 2–10 seconds of signal integration.
4. Pipet samples* (5–20 µl 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.

* 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 cells. 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-gal activity assays.

Figure 5: *Gaussia* Luciferase assays.



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.

Equilibration of the GLuc assay solution is not necessary.

After adding the GLuc assay solution to the sample, we recommend a delay time of 5–15 seconds before taking a measurement. Keeping the delay time consistent across experiments will ensure reproducibility.

Use the prepared 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 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 white or black plates 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.

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.

Ordering Information

PRODUCT	NEB #	SIZE
BioLux® *Gaussia* Luciferase Assay Kit	E3300S/L	100/1,000 assays
COMPANION PRODUCTS		
BioLux® *Gaussia* Luciferase Flex Assay Kit	E3308S/L	100/1,000 assays
pNEBR-X1GLuc Control Plasmid	N8080S	20 µg
pCMV-GLuc Control Plasmid	N8081S	20 µg
pGLuc-Basic Vector	N8082S	20 µg
pTK-GLuc Vector	N8084S	20 µg
pGLuc Mini-TK Vector	N8086S	20 µg
TransPass™ D1 Transfection Reagent	M2553S	0.5 ml
TransPass™ D2 Transfection Reagent	M2554S	0.5 ml
TransPass™ COS/293 Transfection Reagent	M2557S	1.2 ml
TransPass™ HUVEC Transfection Reagent	M2558S	1.8 ml
Luciferase Cell Lysis Buffer	B3321S	25 ml
Anti-GLuc Antibody	E8023S	0.2 ml

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Version 1.1
7/11