

REPORTER SYSTEMS

BioLux[®]
Gaussia Luciferase Flex Assay Kit

Instruction Manual

NEB #E3308S/L
Store at –20°C

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



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Kit Includes:

	#E3308S	#E3308L
BioLux® GLuc Flex Assay Buffer (1X).....	5 ml	2 x 25 ml
BioLux® GLuc Flex Stabilizer.....	0.8 ml	8 ml
BioLux® GLuc Flex Substrate (100X)	0.05 ml	0.5 ml

(Kit components are not sold separately)

Storage Information:

BioLux GLuc Flex Assay Buffer and Stabilizer can be stored at 4°C.
BioLux GLuc Flex Substrate must be tightly capped and stored at -20°C.

Method Overview:

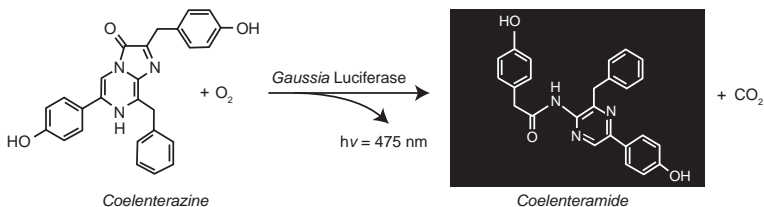
The BioLux® *Gaussia* Luciferase Flex Assay Kit contains reagents necessary for assaying *Gaussia* Luciferase activity with adjustable light emission kinetics, most commonly secreted from mammalian cells transfected with GLuc expression plasmids.

The BioLux GLuc Flex Assay Kit includes a higher concentration coelenterazine substrate and an additional stabilizer component, which allows the use of the assay in high throughput format or without the requirement of an injector-equipped luminometer. With the standard protocol 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). 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 stability.

Gaussia Luciferase is a luciferase reporter from the marine copepod *Gaussia princeps* (1,2). This luciferase, which does not require ATP, catalyzes the oxidation of the substrate coelenterazine in a reaction that produces light, and has considerable advantages over other luminescent reporter genes.

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 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).

Advantages:

***Gaussia* Luciferase (GLuc) 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 than Firefly and *Renilla* Luciferases, making it an ideal transcriptional reporter (1).**

GLuc shows the highest reported activity of any characterized luciferases (2).

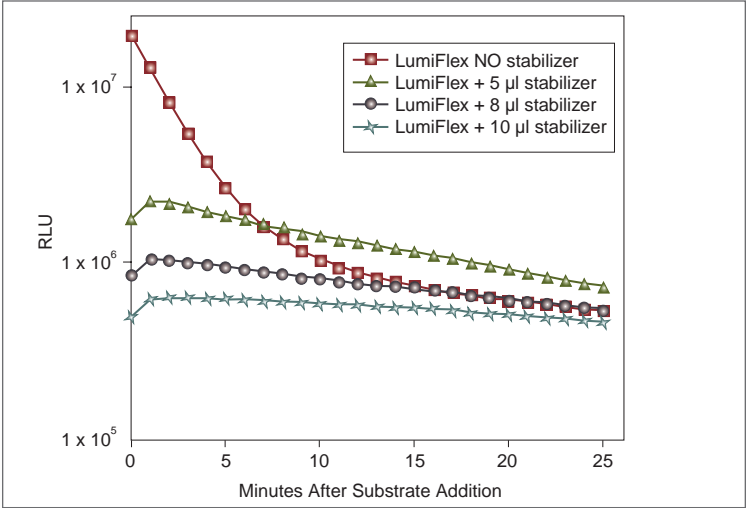
The secreted protein is thermally stable (Figure 3) and has extremely high activity in light production allowing for very sensitive assays (2).

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 after transfection) can be stored at -20°C for long-term storage or at 4°C for several days without loss of activity.

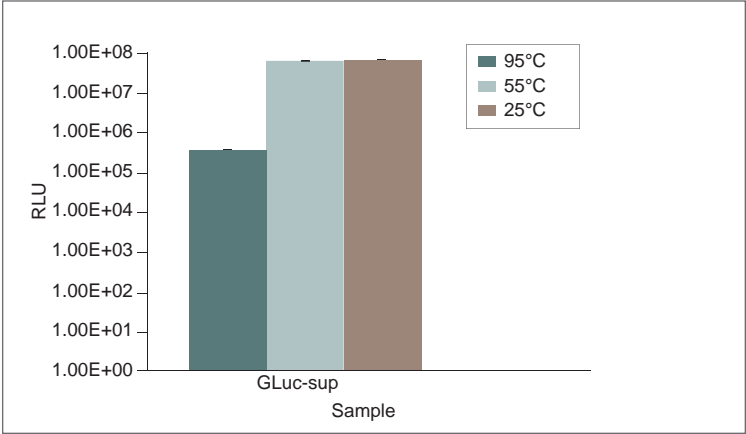
The stabilizer component of this assay system provides steady kinetics over a longer time period allowing users the time required for high-throughput analysis as well as manually delivered assays.

Figure 2: GLuc kinetics with the BioLux GLuc Flex Assay Kit



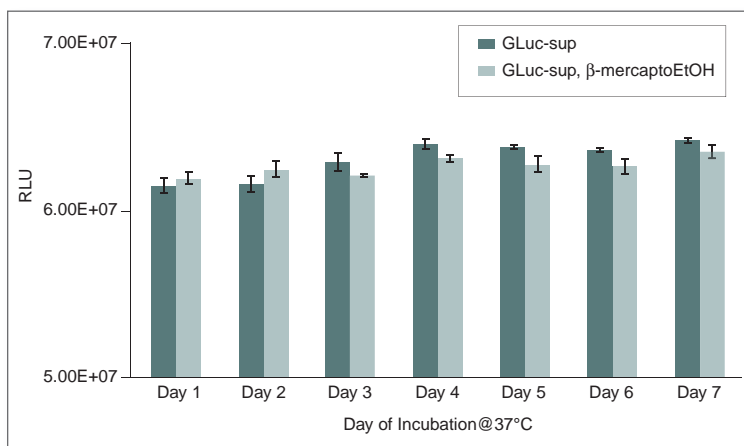
Reactions were setup without stabilizer and with the indicated amounts of stabilizer added. Time is shown in minutes after substrate addition in a total volume of 50 µl.

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 *Gussia* Luciferase at 37°C over a period of 7 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 7 days.

BioLux GLuc Flex Assay Kit Protocols:

Protocol I (Luminometers without injectors):

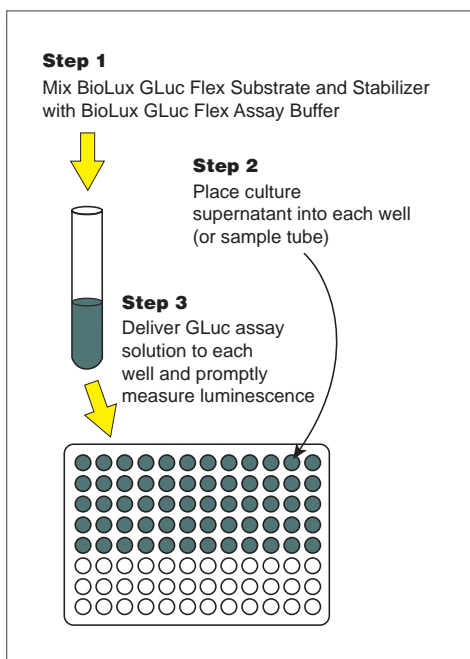
1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 μ l of BioLux GLuc Flex Substrate and 0.8 ml of BioLux GLuc Flex Stabilizer to 5 ml of BioLux GLuc Flex Assay Buffer.
2. Mix well by inverting the tube several times (Do not vortex).
3. Incubate at room temperature for 25 minutes (protect 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 white (opaque) or black plate, or a luminometer 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 Usage Notes) and proceed with the measurement.

Protocol II (Injector-equipped luminometers):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 μ l of BioLux GLuc Flex Substrate and 0.8 ml of BioLux GLuc Flex Stabilizer to 5 ml of BioLux GLuc Flex 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).
 4. Set the luminometer with the 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 white (opaque) or black plate, or a luminometer 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 -gal activity assays.

Figure 5: *Gaussia* Luciferase assays.



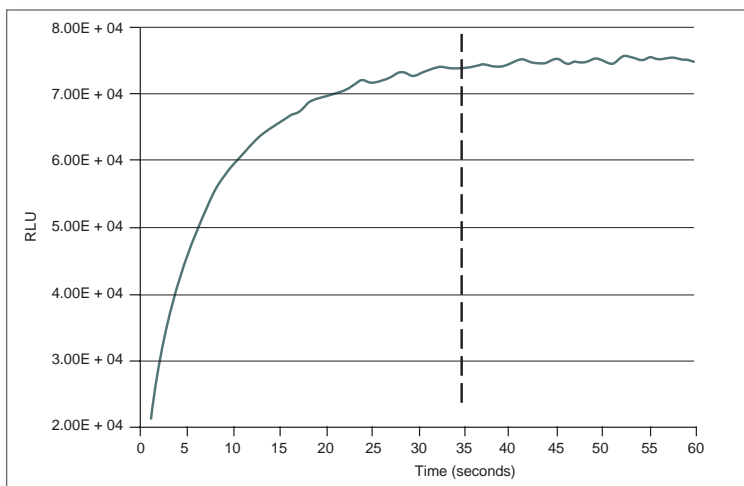
Usage Notes:

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.

The GLuc assay solution should be allowed to equilibrate 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 the maximum level of detection. This is especially important when the GLuc activity level is low (e.g. $< 10^4$ RLU). For example, the readout obtained after 35–40 seconds of delay is $\sim 10^4$; when compared to 30, 20 and 10 seconds of delay, the detection level is $\sim 98\%$ for 30 seconds of delay, $\sim 93\%$ for 20 seconds of delay & $\sim 80\%$ for 10 seconds of delay (Figure 6).

Figure 6: *Gaussia* activity after adding GLuc Flex assay solution to the sample.



The GLuc Flex assay solution was prepared as suggested in the protocol. The measurements were taken at 1-second increments.

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 at 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 (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 either 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 Flex Assay Kit be used without the addition of the Stabilizer?

Yes. If you do not have the BioLux GLuc Flex Stabilizer in the assay solution, the signal obtained will be higher than that obtained from the standard *Gaussia* Luciferase Assay Kit (NEB #E3300). But the signal will show rapid decay (Figure 2), so an injector-equipped luminometer is highly recommended.

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 GLuc Flex 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® <i>Gaussia</i> Luciferase Flex Assay Kit	E3308S/L	100/1,000 assays
COMPANION PRODUCTS		
BioLux® <i>Gaussia</i> Luciferase Assay Kit	E3300S/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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