

## BioLux® Gaussia Luciferase Assay Kit



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Lot: 0071512

Exp: 6/16

### Standard Assay 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.

### Standard Assay 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.

### Stabilized Assay Protocol I (Luminometers without injectors):

1. Prepare the GLuc assay solution (e.g. 100 samples) by adding 50 µl of BioLux GLuc Substrate and 800 µl 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 µl per well) into a 96-well plate (opaque, white or black) or a tube.
6. Add the assay solution (50 µl 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 µl of BioLux GLuc Substrate and 800 µl 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 µl of injection, 35–40 seconds of delay (refer to Usage Notes), & 2–10 seconds of integration.
5. Pipet samples\* (5–20 µl 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 ~e4; when compared to 30, 20 and 10 seconds of delay, the readouts are as follows: ~2% decrease (for 30 seconds of delay), ~7% decrease (for 20 seconds of delay), & ~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.



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Version 1.0 11/14

