

Gaussia Luciferase (GLuc) Reporter Assay

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Abbreviation:

- GLuc: Gaussia Luciferase
- Kit is from New England Biolabs, Inc. #E3300S 100 assays / #E3300L 1,000 assays

Kit Components:

- **GLuc Buffer** (1X): It should be stored in aliquots (**500ul/tube**) and is stable at -70°C in the dark.
- **GLuc Substrate** (100X): It should be stored in aliquots (**5ul/tube**) and is stable at -70°C in the dark.

Protocol:

1. Prepare the GLuc assay solution by adding 5 μ l of BioLux GLuc Substrate and **80 μ l of BioLux GLuc Stabilizer** to 0.5 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*(50 μ l per well, 24 well plate) into a luminometer tube.
6. Add the **GLuc assay working solution** (20 μ l) to a sample (i.e. Add the assay solution to only one sample at a time) and promptly measure the luminescence.
7. **Incubate at room temperature for 35–40 seconds** (refer to Notes) and proceed with the measurement.
8. Repeat Step 5 for all samples.

COMMENTS:

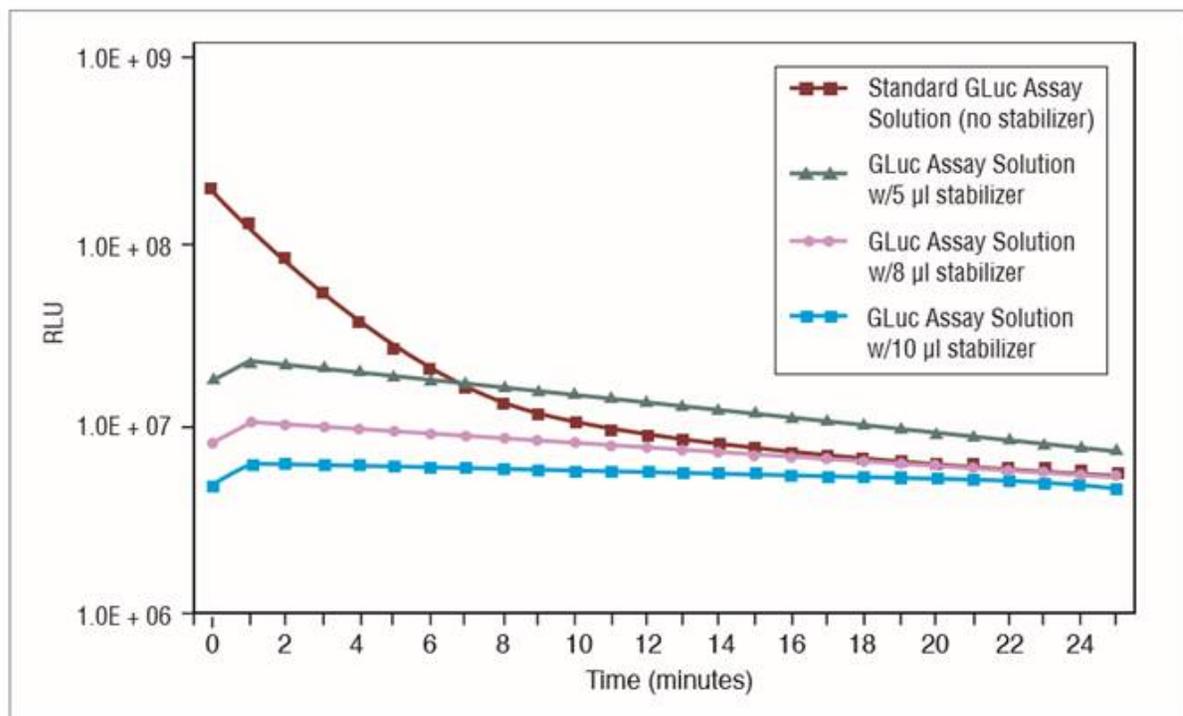
Approximately 90% of GLuc is secreted out into the growth media after transfection and thus, the GLuc activity is typically assayed from the supernatant (i.e. growth media of GLuc-transfected cells). However, as long as the cells are alive, approximately 10% of GLuc is present inside the cells. Therefore, 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.

NOTES:

1. 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.
2. Equilibration of the GLuc assay solution is not necessary.
3. 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.

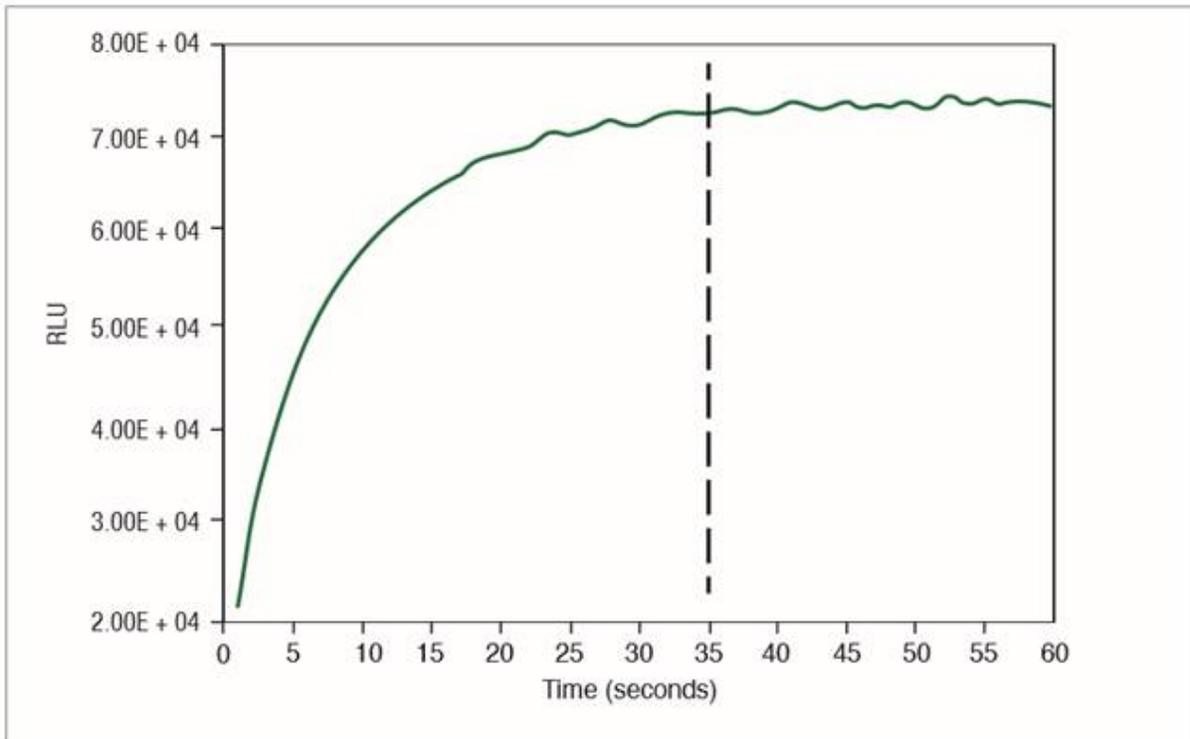
4. Use the prepared assay solution within 24 hours .The unused portion of the assay solution should be tightly capped and stored at -20°C .It should be completely thawed (in the dark) to room temperature before use.
5. The linear range of the luminometer used for the assay must be established. This is 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.
6. If excess activity for the instrument range is found, the sample should be diluted in PBS or 10% serum-containing media.
7. 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.

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).

Figure 5: *Gaussia* 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).