

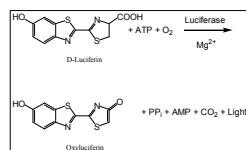
Two New High Quality Reagents for Luciferase Reporter Gene Assays

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1 Introduction



Luciferase from the North American firefly (*Photinus pyralis*) is one of the most frequently used enzymes for reporter gene assays. Firefly luciferase catalyzes the oxidation of the firefly-specific substrate D-luciferin to produce light:



This reaction is extremely efficient and the quantum yield is the highest of any characterized bioluminescent reaction. The bright light produced, makes firefly luciferase a valuable enzyme for reporting promoter activity.

2 Luciferase Assays in HTS

In nature, the firefly luciferase reaction will result in a sharp burst of light followed by a rapid decrease. These so-called 'flash-type kinetics' are less optimal for High Throughput applications because the short period of produced light requires the use of luminometers with injectors, preventing batchwise processing of a large amount of samples.

Over the years, different commercial products have been introduced for luciferase reporter gene assays that generate a longer lasting signal with half-lives ranging from 30 minutes to up to 5 hours. This avoids the necessity of injectors and allows automated batch-wise processing in HTS labs. The true homogeneous nature of these reagents and the ease of operation ('mix-and-measure') has facilitated the rapid adoption of this technology in current Drug Discovery processes.

3 steadyite plus and briteite plus

steadylite plus and briteite plus are luciferase assay systems based on proprietary formulations that modify the enzymatic reaction to produce a longer lasting light output at high signal intensity. However, both reagents do not contain smelly compounds like dithiothreitol (DTT) that are often used to improve the reaction kinetics.

> **steadylite plus** is optimized to give a luminescence signal with a half life of around 5 hours resulting in a detection limit in the femtogram range.

> **briteite plus** produces light with a half life of around 30 minutes at a signal intensity that is approx. 5x higher than steadylite plus resulting in a sub-femtogram detection limit.

Product features:

- High assay sensitivity and long lasting signal
- True mix-and-measure --- ideal for HTS batch-processing
- Odor free!
- Excellent stability → convenient storage conditions (2 - 8 °C)

4 Materials and Methods

steadylite plus: PerkinElmer Cat No. 6016751 (100 mL), 6016752 (10 mL), 6016757 (500 mL), 6016759 (1,000 mL). briteite plus: PerkinElmer Cat No. 6016761 (100 mL), 6016766 (10 mL), 6016767 (500 mL), 6016769 (1,000 mL). Reconstitute one vial of lyophilized substrates with the appropriate volume of substrate buffer solution, as recommended in the user manual. Store unused reagent at -80°C.

The reporter gene assays were performed in White CulturPlate-96 (PerkinElmer Cat No. 6005680, or CulturPlate-384 (PerkinElmer Cat No.6007680), or 1536 white TC-treated plates (Greiner Cat No.782080) microplates.

The protocol used was as follows:

- > Add manually 50 µL (in 96-well plate) or pre-diluted compounds to the cells (12.5 µL in 384-well plate or 1.5 µL in 1536-well plate)
- > Incubate for 4 hours at 37°C
- > Adapt plates to room temperature for 30 minutes. During that time, reconstitute substrate in substrate buffer solution
- > Add manually 50 µL (in 96-well plate) of luciferase substrate (25 µL in 384-well plate and 3 µL in 1536-well plate), slightly tap the edge of the plate to mix components
- > Read at 5 minutes for briteite plus or at 15 minutes for steadylite plus (3 minutes dark adaptation followed by 1 sec reading per well on Topcount, 3 minutes dark adaptation followed by 10 sec reading per well on EnVision).

5 Sensitivity of lite plus Products

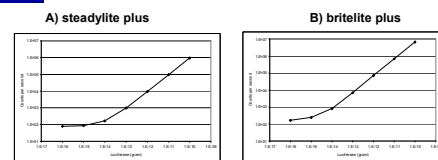


Figure 1. Sensitivity of lite plus products. Dilution series of firefly luciferase enzyme in Dulbecco's PBS/0.1% BSA (100 µL per well) using A) steadylite plus or B) briteite plus in a white 96-well OptiPlate™ (PerkinElmer) measured with the PerkinElmer TopCount® NXT Microplate Scintillation and Luminescence Counter. As can be seen, steadylite plus and briteite plus allow for detection of very low levels of luciferase (femtogram range and low femtogram range for steadylite plus briteite plus, respectively) with excellent linearity.

6 Stability of Lyophilized steadylite plus

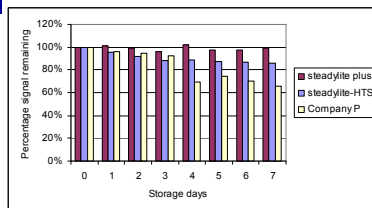


Figure 2. Stability of lyophilized steadylite plus, steadylite-HTS and company P substrate at ambient temperature. All three lyophilized substrates were stored at 22°C for up to 7 days. Over the week, steadylite plus fully retained its activity, contrary to the steadylite-HTS and company P products, which generated 15% and 35% less signal at day 7, respectively.

7 Stability of Lyophilized briteite plus

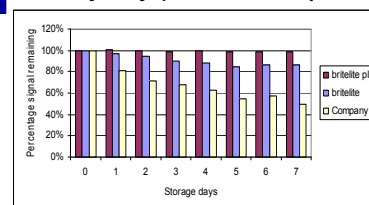


Figure 3. Stability of lyophilized briteite plus, briteite and company P substrate at ambient temperature. All three lyophilized substrates were stored at 22°C for a period of 7 days. briteite plus did not show any decrease in activity over the period tested, while briteite and company P product lost 14% and 50% activity over a week, respectively.

8 Reporter Gene Assays using steadylite plus Agonist Dose-Response Curves on β₂-Adrenergic G_s-coupled Receptor

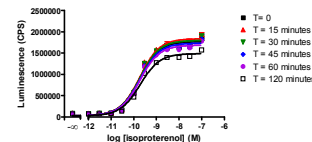


Figure 4. Isoproterenol agonist dose-response curves generated at different incubation times on CHO/6CHL cells expressing the human β₂ adrenergic receptor using steadylite plus reagent. Isoproterenol stimulated luciferase production with an average EC₅₀ value of 0.19 nM. The signal generation was rapid and stable over the 2 hours testing period.

9 Reporter Gene Assays using steadylite plus Antagonist Dose-Response Curves on β₂-Adrenergic G_s-coupled Receptor

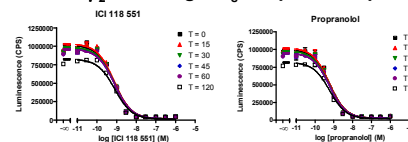


Figure 5. Dose-response curves of two antagonists (ICI 118551 and propranolol) generated at different incubation times on CHO/6CHL cells expressing the human β₂ adrenergic receptor using steadylite plus reagent. Cells were co-treated with isoproterenol (at EC₅₀ concentration) and with increasing doses of antagonists. ICI 118551 and propranolol dose-dependently inhibited luciferase production induced by isoproterenol with expected IC₅₀ values (0.8 and 0.6 respectively). Furthermore, the IC₅₀ obtained in both cases were stable overtime.

10 Reporter Gene Assays using briteite plus 5-HT_{1A} G_i-Coupled Receptor

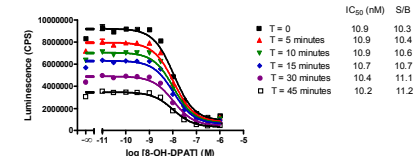


Figure 6. 8-OH-DPAT agonist dose-response curves generated at different incubation times on CHO/6CHL cells expressing the human 5-HT_{1A} receptor using briteite plus reagent. The cells were co-treated with 1 µM forskolin and increasing concentrations of the agonist 8-OH-DPAT. 8-OH-DPAT produced a dose-dependent inhibition of forskolin-stimulated luciferase production with average IC₅₀ values of 11 nM. While counts decreased, IC₅₀ and S/B values remain constant over the 45 minutes period tested.

11 Miniaturization of Luciferase Assays

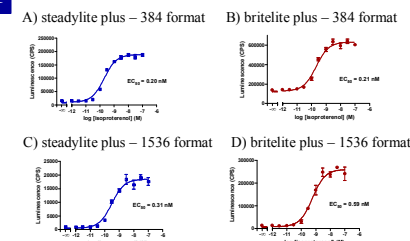


Figure 7. Isoproterenol dose-response curves in 384- and 1536-well formats using steadylite plus and briteite plus products. In both formats, isoproterenol stimulated luciferase production with expected EC₅₀ values.

12 Conclusion

> The data presented here demonstrated that the two new lite plus products are high-performing luciferase reagents ideal for reporter gene assays in 96, 384, and 1536 formats. Both products have equivalent performance to current briteite and steadylite-HTS products.

> Main improvements over existing luciferase reagents include an excellent stability at ambient temperature, allowing convenient storage at 2-8°C for greater flexibility, and the odor-free formulation improving lab atmosphere and eliminating the need for hood work.

> In addition, the lite plus products present the specific following benefits:

steadylite plus:

- High assay sensitivity
- Designed for batch-processing
- Long lasting signal

briteite plus:

- Very high assay sensitivity
- Designed for continuous process systems
- Ideal for smaller-scale sample analysis when maximum sensitivity is needed