

Enhanced detection sensitivity of luciferase reporter gene assays



PerkinElmer

BioTechniques Protocol Guide 2010 (p. 31)

doi 10.2144/000113284

Read the complete protocol online:

www.biotechniques.com/protocols/113284

New reagents and instrumentation have recently been introduced by PerkinElmer to meet the demanding needs of reporter gene assays. The EnSpire™ Multilabel Plate Reader is now available with ultra-sensitive, dual reporter luminescence detection capability and an easy to use graphical user interface. The newly released neolite™ luciferase assay system provides a further increase in sensitivity, while maintaining a signal half-life that enables batch processing of assay plates for medium throughput applications. The performance of EnSpire™ for a luciferase reporter gene assay was demonstrated by measuring a dilution series of firefly luciferase with the neolite™ assay system. In addition, the relative sensitivities and signal half-lives of three PerkinElmer luciferase assays (neolite™, steadylite plus®, and britelite® plus) were compared using a cellular assay.

Materials

EnSpire™ ultra-sensitive luminescence reader (PerkinElmer)

neolite™, steadylite plus®, and britelite plus® luciferase assay systems (PerkinElmer)

Firefly luciferase (QuantiLum™; Promega)

OptiPlate-384 and CulturPlate microplates (PerkinElmer)

For sample sets containing both very high and very low luminescence intensities, crosstalk can be reduced by using the EnSpire™ Reader used with either ray AlphaPlates or black OptiPlates™ (data not shown).

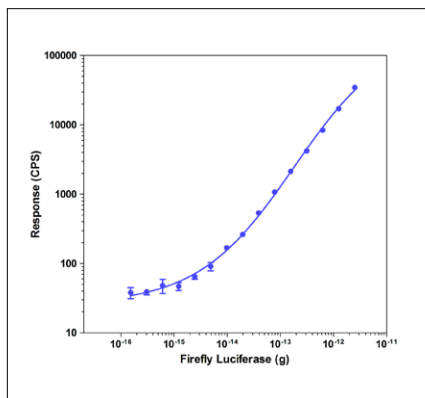


Figure 1. Sensitivity of luciferase reporter enzyme detection. A dilution series of firefly luciferase was measured with the neolite™ high sensitivity luminescence reporter gene assay system in white Optiplate-384 using the EnSpire and 1 s luminescence measurement time.

Protocols

Sample preparation

For luciferase assays: Prepare luciferase dilution series in BSA-containing buffer. Add samples to white OptiPlate-384 microplates in triplicate.

For cellular assays: Seed CCL-64 cells (2000 cells per well) in 384-well white CulturPlate™ microplates and grow for two days in 25 μ L DMEM/10%FBS/1% penicillin:streptomycin without phenol red. neolite™ Luciferase Reporter Assay:

1. Reconstitute neolite™ substrate with reconstitution buffer and store at room temperature.
2. Add neolite™ reagent to each well in a volume equal to the cell culture medium

(25 μ L reagent plus 25 μ L medium for 384-well plates).

3. Mix the samples, seal the plate with TopSeal-A, and incubate 5 min to 2 h. Measure luminescence within 45 min after reagent addition for maximum sensitivity.

4. Read samples using EnSpire™ luminescence setting for OptiPlate-384, with measurement time of 1 s for maximum sensitivity.

Results and discussion

For complete assay detail and full data sets, please download the complete protocol. The combined sensitivity improvements of the EnSpire™ Multilabel Plate Reader and the neolite™ luciferase assay enables the detection of firefly luciferase expression in mammalian cells even when cell numbers are limited or low gene transfection efficiencies are low. Figure 1 illustrates the detection of luciferase activity over a 4 log range of concentrations, detecting sub-attogram levels of the enzyme.

PerkinElmer provides three luciferase assays, tailored to the differing needs of reporter gene assays. The new neolite™ assay provides the greatest sensitivity during its recommended 45 min counting window, and maintains its high sensitivity even after 2 h (Figure 2).

Contact

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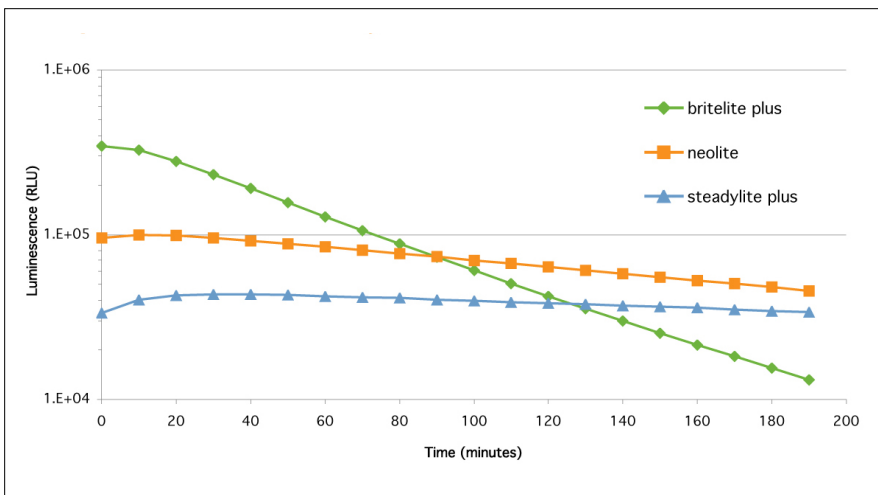


Figure 2. Comparison of luciferase assay performance in a cellular assay. Comparison of signal intensity and half-life for PerkinElmer neolite™, steadylite plus®, and britelite® plus luciferase assays.