britelite plus

Ultra-High Sensitivity
Luminescence Reporter Gene Assay System

PerkinElmer®
precisely.

FOR IN VITRO RESEARCH USE ONLY

Contents	Page
1. Introduction	3
2. britelite™ plus description	4
3. Contents and storage of britelite plus	11
4. Additional requirements	13
5. Assay procedure	13
6. Stability of britelite plus reagent	15
7. Recommendations for use	16
8. Ordering Information	17
9. References	19

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 from the reaction makes firefly luciferase a valuable enzyme for reporting promoter activity.[1,2]
Light emission results from multi-step reactions. The initial reversible reaction step is the activation of D-luciferin in the presence of ATP, Mg^{2+} and luciferase enzyme which leads to enzyme bound adenyl-luciferin with the elimination of inorganic pyrophosphate (PP_i).
Subsequent reaction steps involve the oxidation of adenyl-luciferin with molecular oxygen via adenyl-oxyluciferin to yield AMP, CO_2 and oxyluciferin. The oxyluciferin is generated in an electronically excited state which emits light upon transition to the ground state. The overall reaction is shown in Figure 1.

When light emission is initiated by the addition of luciferase to a reaction mixture containing ATP, Mg^{2+} and D-luciferin in the presence of oxygen, a fast increase in light intensity can be seen followed by a rapid decrease to

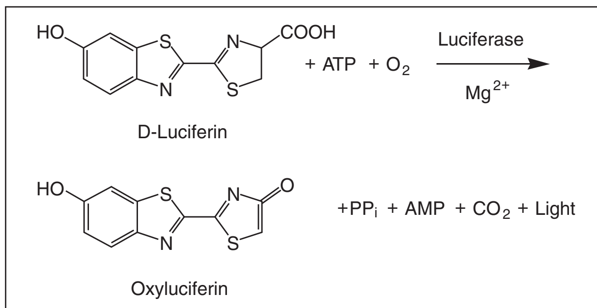

Figure 1: *Reaction scheme*

a low level of sustained light (flash-type kinetics). These flash-type kinetics have been thought to be the result of the formation of intermediate product (adenyl-oxyluciferin) at the enzyme surface which inhibits the enzyme.[4]
Several substances have been described that stimulate the light production by promoting the release of the inhibitor from the enzyme.[3,4] The enhancement in enzyme turnover yields an increase in light output.
The PerkinElmer britelite plus assay system is a proprietary formulation that modifies the enzymatic reaction to produce a longer lasting light output at high signal intensity.

2. britelite plus description

britelite plus is a homogeneous long-lived glow type high

sensitivity firefly luciferase reporter gene assay system for the quantification of firefly luciferase expression in mammalian cells. The reagent formulation contains compounds that facilitate both cell lysis as well as the luciferase enzymatic reaction. britelite plus is especially suitable for continuous process systems using microplates in ultra-high throughput environments. These in-line systems do not require a very long signal half-life since the time between addition of the reagent and reading the resulting luminescence is relatively short (minutes). In general, britelite plus has a signal half-life of approximately 30 minutes. This relative short half-life has facilitated the design of this luciferase assay system with a very high light output. britelite plus does not contain thiol compounds like dithiothreitol (DTT) and is therefore odor free.

britelite plus offers the following benefits:

- Very high assay sensitivity
- Designed for continuous process systems
- Suitable for higher density microplates such as 384-well and 1536-well plates
- Ideal for smaller-scale sample analysis when maximum sensitivity is desired
- Odor free
- Convenient storage conditions (2 - 8 °C)

The very high light output of britelite plus is proportional to the firefly luciferase concentration in the sample and allows detection of very low levels of luciferase in microplate formats. Figure 2 shows the assay result of a dilution series of firefly luciferase enzyme in Dulbecco's PBS/0.1% BSA (100 μL per well) using britelite plus in a white 96-well OptiPlate™ (PerkinElmer) measured with the PerkinElmer TopCount® NXT Microplate Scintillation and Luminescence Counter. As can be seen, britelite plus allows for detection of very low levels of luciferase (low femtogram range) with excellent linearity.

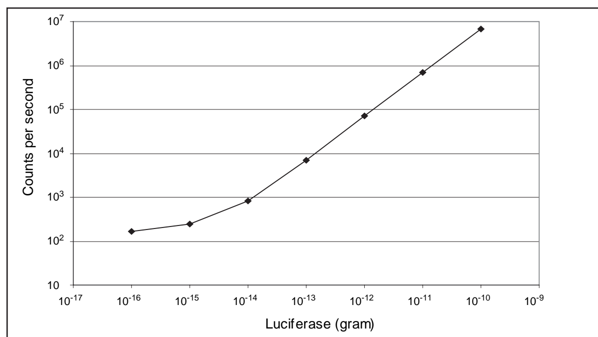

Figure 2: *Sensitivity of britelite plus*

Figure 3 highlights the time course of the light output of the described experiment above for 10^{-10} gram luciferase per well. After britelite plus addition, an incubation time of up to 5 minutes is needed for full signal generation.

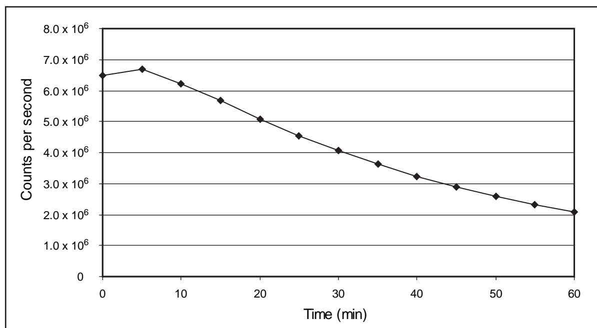

Figure 3: *Time course of the luminescent signal of britelite plus*

To assess the influence of different culture media on luminescence light output, the following experiment was performed using commercially available basal media in the presence and absence of Fetal Bovine Serum (FBS) and Phenol Red (PR). 100 μL britelite plus was added to 100 μL samples of the different media in a white 96-well OptiPlate, where each sample contained luciferase at a concentration of 5 x 10^{-10} gram per mL. After shaking the plate, the light output was measured using a TopCount NXT. Figure 4 illustrates the results of the relative luminescence after 5 minutes count delay. Figure 5 shows the results of the stability of the signal as a function of the culture media used.

The results from Figure 4 show that the light output is to some extent affected by FBS but that Phenol Red has a much larger impact on the light output reflecting the concentrations of Phenol Red in the basal media. Consequently the use of Phenol Red in culture media should be avoided to attain the highest signal. The stability of the luminescent signal is only somewhat dependent on the media as shown in Figure 5.

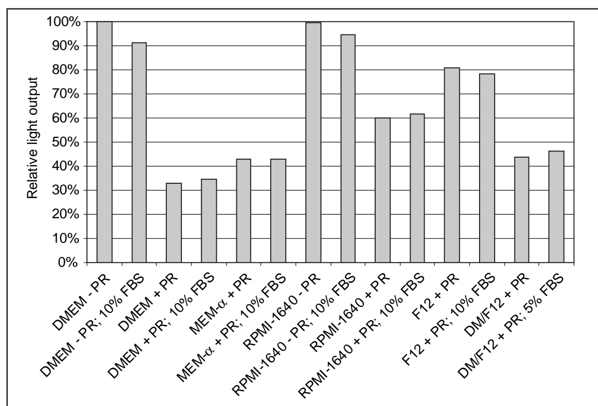

Figure 4: *Relative light output in different media*

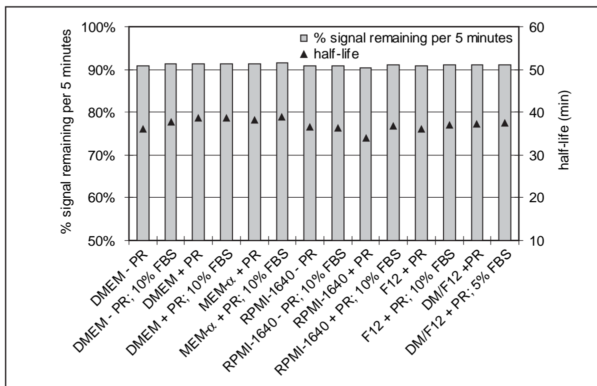

Figure 5: *Signal stability in different media expressed as a percentage of signal remaining per 5 minutes and half-life (minutes)*

Organic solvents are often used to introduce screening compounds, resulting in the presence of a small percentage of organic solvent in the culture medium. The effect of organic solvents on the light output of britelite plus was investigated in the following experiment. Luciferase in culture medium (DMEM without Phenol Red, supplemented with 10% FBS) was added at 100 μL per well to a white 96-well CulturPlate™ (PerkinElmer). The medium contained various concentrations of organic solvents (DMSO, ethanol, isopropanol and acetonitrile). Next, 100 μL of britelite plus was added to the wells. After shaking, the plate was sealed and the luminescence was

measured using TopCount NXT. The results presented in Figure 6 show that DMSO and ethanol do not affect the signal at the indicated solvent concentrations. A decrease in signal was shown for the other solvents which was dependent on the specific solvent concentration. The stability of the signal as shown in Figure 7 is not compromised when using these four solvents at the tested concentrations.

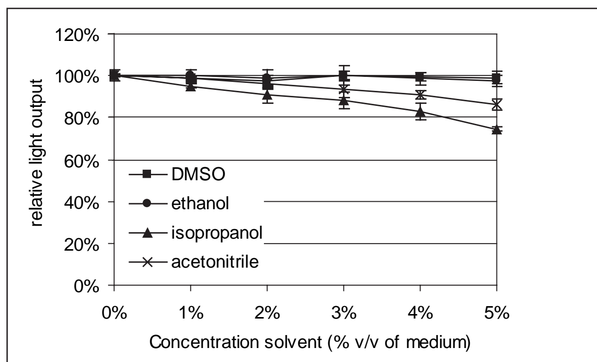

Figure 6: *Relative light output in the presence of organic solvents*

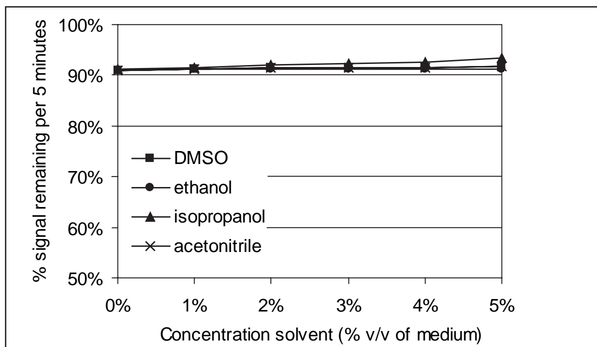

Figure 7: *Signal stability in the presence of organic solvents as a percentage of signal remaining per 5 minutes*

3. Contents and storage of britelite plus

6016766 – britelite plus 10 mL kit

Each 10 mL kit contains the following components:

- 1 vial britelite plus Lyophilized Substrate
- 1 bottle britelite plus Reconstitution Buffer
- Manual

6016761 – britelite plus 100 mL kit

Each 100 mL kit contains the following components:

- 10 vials britelite plus Lyophilized Substrate
- 1 bottle of britelite plus Reconstitution Buffer
- Manual

6016767 – britelite plus 500 mL kit

Each 500 mL kit contains the following components:

- 10 vials britelite plus Lyophilized Substrate
- 1 bottle of britelite plus Reconstitution Buffer
- Manual

6016769 – britelite plus 1000 mL kit

Each 1000 mL kit contains the following components:

- 4 bottles britelite plus Lyophilized Substrate
- 2 bottles britelite plus Reconstitution Buffer
- Manual

Number of data points per part number*

britelite plus part number	kit size	96-well plate	384-well plate	1536-well plate
6016766	10 mL	100	400	3,300
6016761	100 mL	1,000	4,000	33,000
6016767	500 mL	5,000	20,000	165,000
6016769	1000 mL	10,000	40,000	330,000

* Based on recommended volumes per well:
100 μL for 96-well, 25 μL for 384-well and 3 μL for 1536-well plate

Storage conditions:

britelite plus is shipped at ambient temperature and must be stored at 2 - 8 °C upon receipt.

For convenience, the britelite plus Reconstitution Buffer can be stored separately at room temperature (max. 22 °C). This allows addition of the Reconstitution Buffer to the Lyophilized Substrate without the need to equilibrate to room temperature.

If stored at the recommended conditions, the kit components are stable through the expiry date found on the kit label.

4. Additional requirements

A detection instrument such as the PerkinElmer TopCount, MicroBeta®, VICTOR™ Light, VICTOR^3 Multi Label Reader or EnVision™ is required. CCD camera systems, such as PerkinElmer ViewLux™ can be used for high throughput applications.

For optimum light yield, low background and minimum well-to-well crosstalk, white microplates should be used. We recommend the use of the PerkinElmer CulturPlate, OptiPlate or ViewPlate® (when visual inspection of cells is preferred). Black plates can also be used when very high signals are expected. Black plates will reduce well-to-well crosstalk but will also quench the light output. See section 8 for a complete list of recommended plates with part numbers.

5. Assay procedure

1. Equilibrate the kit components to room temperature (20 - 22 °C) before reconstitution.

2. For the **10 mL** and the **100 mL** kit reconstitute one vial of britelite plus Lyophilized Substrate with **10 mL** of britelite plus Reconstitution Buffer.

   For the **500 mL** kit reconstitute one vial of britelite plus Lyophilized Substrate with **50 mL** of britelite plus Reconstitution Buffer.

   For the **1000 mL** kit reconstitute one bottle of britelite plus Lyophilized Substrate with **250 mL** of britelite plus Reconstitution Buffer.

   Mix the contents of the vial gently by inversion and leave for 5 minutes. This should result in a clear homogeneous solution.
   Keep the britelite plus reagent at room temperature (20 - 22 °C) before use.

3. Only prepare as much britelite plus reagent as needed for one day.

4. Add britelite plus reagent to each well. Equal volumes of cell culture medium and britelite plus reagent should be used.

   For **96-well** plates: add **100 µL** to each well containing **100 µL** of cells in medium.

For **384-well** plates: add **25 µL** to each well containing **25 µL** of cells in medium.

For **1536-well** plates: add **3 µL** to each well containing **3 µL** of cells in medium.

5. Mix the well contents (see section 7).

6. Seal the plate with self-adhesive TopSeal™-A.

7. For complete cell lysis and full signal generation wait at least 1 minute, but not longer than 15 minutes.
8. Measure luminescence within 15 minutes after reagent addition for maximum sensitivity.

6. Stability of britelite plus reagent

Stability of reconstituted britelite plus is approximately:

> 85 % remaining signal after 8 hours at 20 °C
> 95 % remaining signal after 8 hours at 4 °C
> 90 % remaining signal after 24 hours at 4 °C
> 80 % remaining signal after 48 hours at 4 °C

Freshly prepared reagents can be aliquoted and stored for one month at -20 °C and for three months at -80 °C.

The reagents can be subjected to at least 10 freeze - thaw cycles without significant loss of activity.

7. Recommendations for use

- Mixing of culture medium and reagent is vital to obtain low coefficients of variation between replicates. This is especially important with 384- and 1536-well plates. Optimize liquid handling procedures to attain optimal reagent/medium mixing. For detailed background information see reference 5.

- Phenol Red, as well as other colored compounds, will chemically not interfere with the luciferin/luciferase reaction, but will quench some of the emitted light, resulting in lower assay signals (see Figure 4). For optimal light output, the culture medium can be substituted with **Dulbecco's PBS containing calcium and magnesium** ions prior to the addition of britelite plus.

- The luciferase reaction requires magnesium ions. Although britelite plus does contain these ions it is strongly recommended that the sample contains sufficient amount of these ions. Normal culture media contain these ions at sufficient levels.

- When handling the plates prior to measurement, work under subdued light conditions and avoid direct sunlight or bright fluorescent light. Bright light may cause plate phosphorescence resulting in higher background levels. Plate phosphorescence has a half-life of several minutes.

- If more than one vial of substrate is reconstituted, it is advised to pool all reagents before addition to the plates.

- Optimal room and instrument temperature is 22 °C. Allow plates to adapt to room temperature after removal from the incubator and prior to the addition of the reagent. An adaptation time of 30 minutes is usually sufficient. Stacked plates will need more time to adapt to room temperature.

8. Ordering Information

britelite plus	Reorder No.
10 mL britelite plus assay kit	6016766
100 mL britelite plus assay kit	6016761
500 mL britelite plus assay kit	6016767
1,000 mL britelite plus assay kit	6016769

Accessories	Reorder No.
CulturPlate-96, white, TC /case of 50	6005680
CulturPlate-384, white, TC /case of 50	6007680
CulturPlate-96 F, black, TC /case of 50	6005660
CulturPlate-384 F, black, TC /case of 50	6007660
OptiPlate-96, white /case of 50	6005290
OptiPlate-384, white /case of 50	6007290
OptiPlate-96 F, black /case of 50	6005270
OptiPlate-384 F, black /case of 50	6007270
OptiPlate-1536, white /case of 20	6005228
OptiPlate-1536 F, black /case of 20	6005235
ViewPlate-96, white, TC /case of 50	6005181
ViewPlate-384, white, TC /case of 30	6005262
ViewPlate-96, black, TC /case of 50	6005182
ViewPlate-384, black, TC /case of 30	6005261
TopSeal A, 96-well, self adhesive /100 pcs	6005185
TopSeal A, 384-well, self adhesive /100 pcs	6005250

For further information on luminescence readers, microplates or luminescence applications please contact your local PerkinElmer representative or visit: http://www.perkinelmer.com.

9. References

1. Alam, J. and Cook, J.L. (1990). Reporter genes: application to the study of mammalian gene transcription. Anal. Biochem. 1990 Aug 1; 188(2) 245-254

2. Collin Goddard (1994). Cell based screening approaches: advantages of highly automated robotics technology in HTS. Handbook for The 1994 International Forum on Advances in Screening Technologies and Data Management, p.19

3. Wood, K.V. Recent advantages and prospects for use of beetle luciferases as genetic reporters. In: Bioluminescence and Chemiluminescence current status. Proceedings of the VIth International Symposium on Bioluminescence and Chemiluminescence, Cambridge, September 1990. p. 543. Ed. by P. Stanley and L.J. Kricka.

4. Airth, R.L., Rhodes, W.C. and McElroy, W.D. (1958). The functioning of Coenzyme A in Luminescence. Biochemica and Biophysica Acta. Vol. 27, pp. 519-532

5. Hancock M.K., Medina M.N., Smith B.M. and Orth A.P.. Microplate Orbital Mixing Improves High-Throughput Cell-Based Reporter Assay Readouts. J. Biomol. Screen. 2006 Nov 27. 2 2007; vol. 12: pp. 140 - 144

This product and/or the use of this product are covered by pending PerkinElmer patent applications. By purchasing this product the end user is granted a limited license to use the britelite plus kit and reagents for research purposes. Purchase does not include any right to use, develop or otherwise exploit this product commercially.

Limited Use License

This product is distributed and sold for life science research and commercial applications, but not for diagnostic use. Any use of this product other than for life science research and commercial applications is strictly prohibited.

Rev. A - March 2007

PerkinElmer Life and Analytical Sciences
710 Bridgeport Avenue
Shelton, CT 06484-4794 USA
Phone: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

For a complete listing of our global offices, visit www.perkinelmer.com/lasoffices.

0RD1100

BRTLTPL-1206 / REV. A

© 2007 PerkinElmer, Inc