Live-cell luciferase assay of drug resistant cells

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Introduction

To date, multiplexing cell-based assay is essential for high-throughput screening of molecular targets. Measuring multiple parameters of a single sample increases consistency and decrease time and cost of assay. Functional assay of living cell is useful as a first step of multiplexing assay, because live-cell assay allows following second assay using cell lysate or stained cell. However, live-cell assay of drug resistant cells that are highly activated of drug efflux mechanisms is sometimes unstable or difficult; for example, the method of measuring colored formazan products by living cells does not show correlation between the amount of formazan and the cells numbers. To this end, more reliable method to allow live-cell assay is anticipated.

We described here the protocol of live-cell luciferase assay as a first step of multiplexing cell-based assay of drug-resistant cells which expresses firefly luciferase. This method has several advantages; 1) In growth assay of P-glycoprotein-overexpressing multidrug-resistant cells, firefly luciferase bioluminescence from live cells correlates with the cells numbers; 2) Live-cell assay allows performing second assays, such as a caspase assay, Hoechst staining, and a cell-direct real-time RT-PCR, using the same cells. If necessary, it is possible to continue the cell culture replacing the assay solution with fresh medium; 3) Live-cell luciferase assay is available for other mammalian cells besides drug-resistant cells.

Reagents

Cells: We used bioluminescent MCF7-ADR-Luc cells that are P-glycoprotein-overexpressing multidrug-resistant beast cancer cells and stably transfected with firefly luciferase gene. MCF7-ADR-Luc cells were cultured in an RPMI 1640 (Gibco BRL) supplemented with 10% fetal bovine serum (Gibco BRL).

Luciferin solution: 1 mM beetl luciferin, potassium salt (Promega, E1602) in cell culture medium. Prepare just before assay and equilibrate to room temperature.

Equipment

- 96-well cell culture plate, white well with clear bottom (BD)
- Multichannel pipette or automated liquid dispenser for addition of Reagent
- Plate shaker for mixing multiwell plates
- Luminescent plate reader or CCD camera imaging device. We used Wallac Multi-label Counter

(PerkinElmer)

Procedure

1. Culture cells in 96-well plate by time point.
2. Equilibrate the plate to room temperature for 30 min.
3. Prepare control wells containing medium without cells for background.
4. Add a volume of luciferin solution equal to the volume of cell culture medium in a well (luciferin solution (vol.) : cell culture medium (vol.) = 1 : 1).
5. Mix the contents of plate by gently shaking for 5 sec.
6. Immediately detect the luminescence with a luminescent plate reader. Suitable measurement time is 1~10 sec depending on equipment type.

Timing

The reagent preparation and measuring procedure will require 1 hour.

Critical Steps

The assay yields a linear correlation between cell number and luminescence: up to 50,000 cells per well for 96-well plates within a measurement window of zero to 15 minutes. Activity of luciferase enzymes diminishes under a wide range of assay conditions, such as variable temperature, varied ionic strength, and prolonged exposure in solution.

Troubleshooting

Low luminescent signal

• Measure immediately after addition of luciferin, since flash-type luminescent rapidly decays.

• Extend measurement time.

Low correlation between the luciferase luminescence and the cells numbers

• Dispense luciferin solution and measure luminescence by well-to-well. In this case, automated liquid dispenser is needed.

• If ABCG2/BCRP-overexpressing cells are used, the assay may be incorrect, since ABCG2/BCRP is a transporter of luciferin[1].

Anticipated Results

The firefly luciferase luminescence from live cells will be directly proportionally to the number of cells

(Figure 1).

References

1. Zhang, Y. et al. ABCG2/BCRP expression modulates D-Luciferin based bioluminescence imaging. *Cancer Res.* 67, 9389-9397 (2007)

Acknowledgements

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Figures

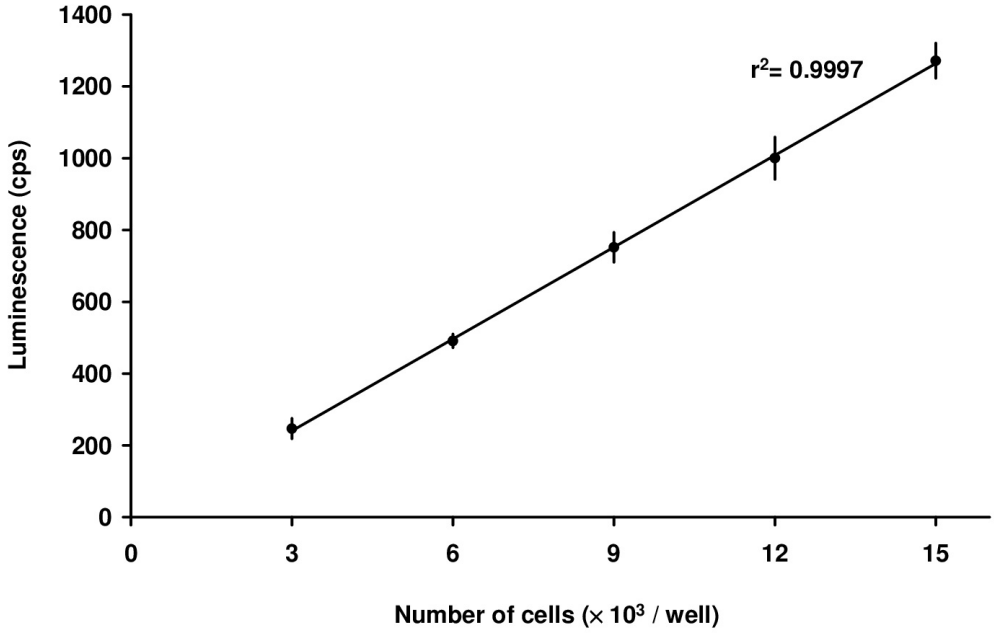

Figure 1

Various numbers of MCF7-ADR-Luc cells were seeded in a 96-well plate, after cell adhesion to the bottom of well, firefly luciferase bioluminescence was measured. Values are means ± s.d. _n_ = 6. The firefly luciferase luminescence from live cells showed directly proportionally to the number of cells (r^2^ = 0.9997).

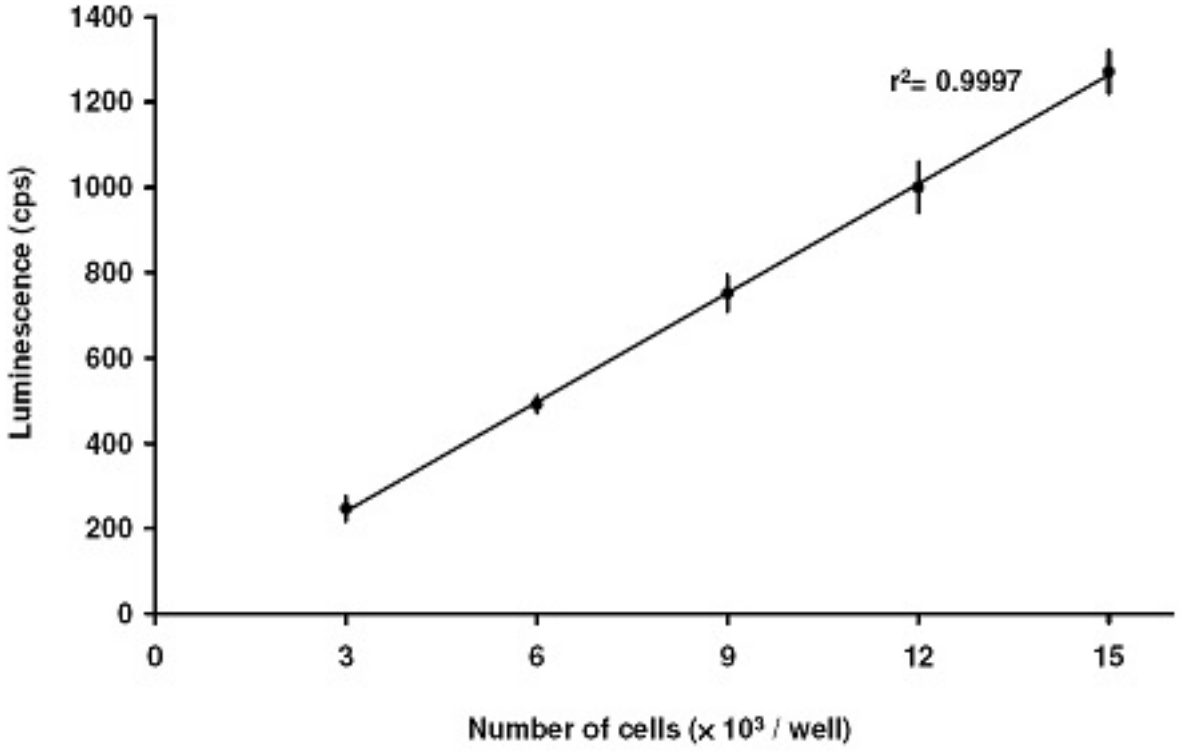

Figure 2

RPN2 gene confers docetaxel resistance in breast cancer

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