

FDSS Cell dispensing unit for Aequorin Flash luminescence

- 24hours HTS using FDSS/overnight runs /Cell injection techniques -

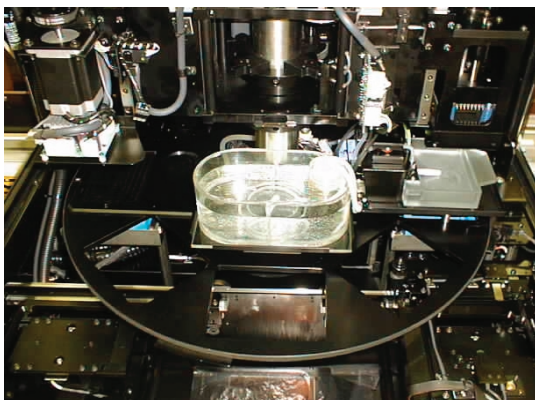
Introduction

Hamamatsu Photonics K.K. provides a high throughput screening (HTS) system called FDSS for cell-based assays. FDSS can be used as a versatile tool for not only fluorescent assay but also luminescent assay in both 96- and 384-well microplates. The luminescent signal detection is accomplished using 2 dimensional photon counting detector even in single photon light level. Because of the single photon light level sensitivity and no time lag from dispensing to light detection, FDSS is the most suitable HTS system for flash Aequorin assay. Here we have validated a new cell dispensing unit containing the Cell vat and Cell tank, for suspension cells injection into 96- and 384-well plates.

Cell dispensing unit (optional)

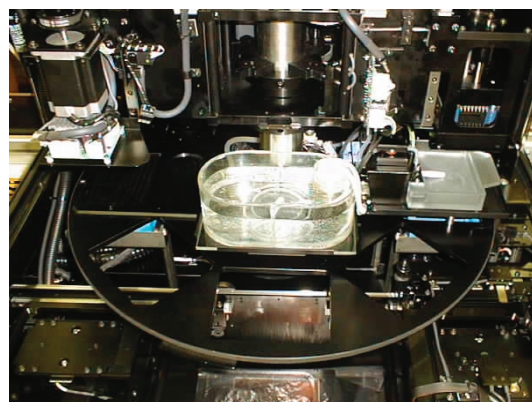
The novel feature in FDSS cell dispensing unit is the inclusion of multiple reagent injectors for measuring prompt (or "flash") reactions. 1000 ml/10 ml (1 plate, 25 μ l/1 well) = 100 plate for over night loading

Cell tank



Cell tank volume 1000 ml

Cell vat



Cell vat dead volume 50 ml

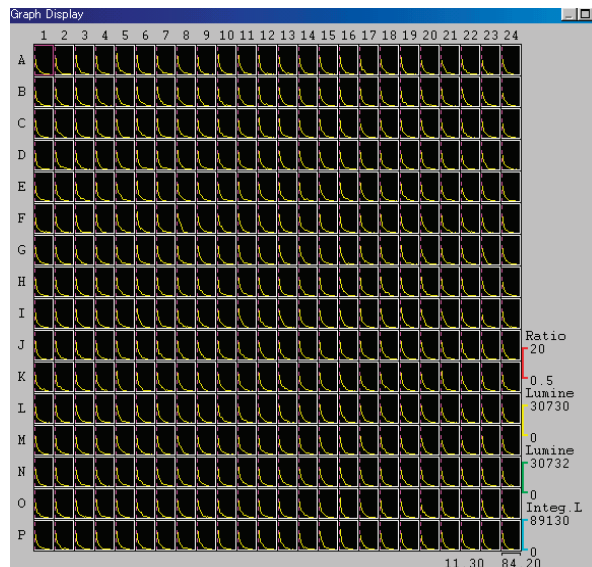
Materials and Methods

The AequoScreen™ cell lines

Aequorin assays have been validated for many GPCRs and calcium channels, and the results are similar to those obtained with fluorescent calcium dyes. The main challenge for adapting aequorin to HTS, however, is the fast measurement of flash luminescence. A typical aequorin signal is emitted in an interval of less than 30 s. Euroscreen (Euroscreen s.a., Brussels, Belgium) previously developed the AequoScreen™, an aequorin-based assay suitable for medium throughput screening and pharmacological characterization. Cell lines (usually CHO-K1, other ones in some cases) were stably transfected with plasmids intended for expression of apoaequorin and of a GPCR. After selection with antibiotics, recombinant cells were subjected to a limit dilution and clones expressing the G protein-coupled receptor and apoaequorin at a high level were selected. If the G protein-coupled receptor is not naturally coupled to a calcium signalling pathway, a universal coupling effector is co-expressed in order to redirect the coupling towards intracellular calcium release. This universal coupling effector is usually the G 16 protein (Milligan et al., 1996), that was shown to be able to couple many GPCRs to the calcium pathway. The aequorin photoprotein undergoes a bioluminescent reaction in the presence of calcium ions, producing a flash of light at 469 nm. This wavelength correlates well with the maximum quantum efficiency of the CCD used in the FDSS.

How to perform the assay

Cells expressing apoaequorin and a GPCR are detached from the culture plate (if they are adherent) and are incubated with coelenterazine to reconstitute active aequorin. These are then maintained in suspension with a stirrer and the cell suspension is injected, well by well, on the solutions of tested compounds. Light emission is then recorded usually for 2030 seconds. By injecting the same cell suspension in each of the 96-/384-wells, this method allows 96-/384- measurements of agonist-induced aequorin light emission in few minutes. FDSS can perform 96-/384- measurements in 5 minutes or up to 30,000 measurements in one day. Once the cells have been incubated with coelenterazine, they can be used for several hours (over 12 hours) and even several days for the measurement of agonist-induced increase of intracellular calcium concentration. A signal-to-noise ratio above 50 was currently obtained with this system of cell injection.



Results and Discussion

The sensitivity of the FDSS was similar to that of PMT based luminometers (Top Counts) for AequoScreen assays, and allows efficient screening in both 96- and 384-well plates, with similar EC50 values. A Z factor average of 0.71 and 0.78 was calculated for 96- and 384-well plates respectively (Le Poul E et al., 2002). Cell injection techniques have several additional advantages, including the control of the number of cells per well, the possibility of mixing several cell lines expressing different receptors, lower costs (as a result of a decrease in consumables), and low well-to-well and plate-to-plate signal variation. The FDSS shows good linear response to change in aequorin concentration and therefore provides a suitable method for studying calcium flux in high throughput screening assays. Here we present the stable data for the use of aequorin in functional HTS with FDSS, a new generation ultrasensitive luminometer equipped with an integrated pipetting system suitable for HTS flash luminescence.

References

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2. Milligan G, Marshall F, Rees S Ga16 as a universal G protein adapter: implications for agonist screening strategies. *Trends Pharmacol Sci* 1996; 17: 235-237.

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