New Microplate Luminescence Counter for Aequorin based Ca2+-coupled GPCR Assays

Authors: Ville-Veikko Oksa, Petri Kivelä, Tiina Lähde, Miika Talvitie

1 Introduction
The AequorinScreen® technology is a generic GPCR technology based on aequorin photoprotein which can be used with Gq, Gs, and Go coupled GPCRs and calcium coupled ion channels. In the aequorin assay, cells co-expressing aequorin and the target receptor are first incubated with the co-factor coelenterazine in order to reconstitute the active aequorin enzyme. Reconstitution of an active aequorin, using native coelenterazine or its derivative coelenterazine h, yields an enzyme having a fast luminescent response to increasing calcium concentrations, and a high level of signal intensity. The aequorin photoprotein undergoes a luminescent reaction in the presence of calcium ions, producing a flash of light peaking at 469 nm. This wavelength correlates well with the maximum quantum efficiency of the PMT’s used in the MicroBeta®.

In this poster we present the Aequorin technology as performed with the MicroBeta® microplate counter, the latest luminescence and liquid scintillation reader from PerkinElmer. Equipped with injectors, the MicroBeta® LumiJET™ is shown to be uniquely suited for flash luminescence assays such as the Aequorin assay.

2 MicroBeta® LumiJET Microplate Counter
MicroBeta® is a multi-detector instrument designed for liquid scintillation or luminescence detection of samples in microwells, strips or on filters. It is available in 1-, 2-, 6-, and 12-detector versions. The 1- and 2-detector models are equipped with 24- and 96-well counting capabilities and by default the 6- and 12-detector units with 96- and 384-well support. MicroBeta® is available with sample capacities of 18 or 32 plates in a robotic loading option.

MicroBeta® LumiJET can be equipped with reagent injectors needed to perform aequorin assays in 384-well plates. The performance of the MicroBeta® LumiJET is highly suitable for a wide range of radiolabel-free-based assays in addition to "flash and glow" type luminescence assays with up to 12 detectors. With two injectors per detector, the MicroBeta® LumiJET offers the ability run aequorin-based GPCR assays in a dual screen mode.

The software of MicroBeta® is familiar to existing MicroBeta users. Enhancements to the instrument have also improved assay performance where now 0.1 sec kinetic data points can be recorded and viewed, resulting in increased resolution and data quality. The kinetic reactions can be viewed during measurement or the data can be exported to PerkinElmer’s AssayPro data analysis package or to other suitable software.

3 Materials and Methods
To test performance of aequorin/Ca2+ measurements in MicroBeta® LumiJET, agonists and antagonist assays in two cell lines were studied. AequorScreen and AequoZen® Frosen cells were used in this study as described below.

Measurements were performed with Histamine H1 AequoScreen (PerkinElmer #ES-390-A) and Muscarinic M5 AequoZen® FroZen cell lines (PerkinElmer #ES-214-AF). The cell density in 384-well format measurements were 5,000 or 6,000 cells per well for Histamine H1 and Muscarinic M5 assays, respectively. Cell harvesting, coelenterazine h (Invitrogen, #C 6780) loading and preparation were done according to instructions presented in the AequoScreen Starter Kit Manual (PerkinElmer). Compound concentration series (20 μL/well) were diluted in 0.1% BSA (Intergen, #4496) containing assay buffer (20-MEM, pH 5.4, Invitrogen #SH3002) and prepared in white opaque OpTifast® 384 well microplates (PerkinElmer, #0907290). The cell suspension was dispensed on the liquids using MicroBeta® LumiJET (fig. 2). Injector tubing was rinsed (ethanol, water and ethanol), as a positive control solution.

Detection of agonist
Histamine (Sigma, #H7250) and acetylcholine chloride (BioChemika, #A2661) were used as an agonist for the Histamine H1 and Muscarinic M5 cell lines, respectively. The concentrations and dilution series having eight replicates were prepared as instructed in the AequoScreen Starter Kit Manual. Light emission was measured kinetically (0.1 s measurement time) and integrated for 25 seconds.

Detection of antagonist
For the antagonist assays, cells were injected (30 μL) into the assay plate with agonist (20 μL) using the MicroBeta® LumiJET. The antagonist dilution series with eight replicates was prepared as instructed in the AequoScreen Starter Kit Manual. Antagonists used for the Histamine H1 cell line were trans-triprolidine and trans-N-Me-Scolopamine (Sigma, #T6554) for the Muscarinic M5 cell line.

Figure 1: Activation of H1 receptor by Histamine (Sigma, trans-H7250) at a concentration of 10 μM, plated with GraphPad Prism®.

4 Dose Response Assays with Histamine H1 AequoScreen Cell Line
The agonist and antagonist dose-response curves (fig. 3, 4) demonstrate the quality of data produced by the MicroBeta® LumiJET. Both the EC50 and IC50 values are in accordance with previously defined values and the Z’ = 0.6 for shows that AequorScreen assays can be run successively with 384-well format using MicroBeta® LumiJET.

5 Dose Response Assays with Muscarinic M5 AequoZen FroZen Cell Line
Both the agonist and antagonist for Muscarinic M5 receptor performed in dose dependent manner. The activation and inhibition assays produced data with high Z’ values and accurate EC50 and IC50 values despite the use of a frozen cell line exhibiting low signal level.

Figure 2: The plate map used for the Histamine H1 Aequorin cell line dose response assay. Dual screen model in MicroBeta® LumiJET enables to acquire data from both agonist and antagonist responses.

6 Kinetic Mode
Kinetik mode can be used to measure agonist and antagonist responses in a single well. The measurements can be performed either continuously or at time intervals. The data can be viewed during measurement or the data can be exported to PerkinElmer’s AssayPro data analysis package or to other suitable software.

7 Evaluating the Working Cell Density
Cell suspension assay was evaluated at a range of cell densities (200 – 1500 cells/well). At cell density of 500 and over, the assay is very robust as the Z’ value is 0.7. Even at lower cell numbers the assays still show a good performance.

Figure 3: Activation of H1 receptor by Histamine, Curve fitting, error bars (SD) plotted with GraphPad Prism®.

Figure 5: Inhibition of H1 receptor by trans-N-Me-Scopolamine, Curve fitting, error bars (SD) plotted with GraphPad Prism®.

These assays were conducted by pre-dispersing Histamine H1 Aequorin cells (prepared as before) together with agonist or antagonist (trans-triprolidine, final concentration 10 μM) on to OptiPlate-384 in 38 replicates. The agonist (histamine, final concentration 20 μM) was dispensed and resulting luminescence was recorded by MicroBeta® LumiJET (total volume, 70 μL).

8 Summary
The MicroBeta® LumiJET introduces new features and possibilities for a variety of assays to be run with throughput and ease. The aequorin assays that are presented here were run with a 12-detector MicroBeta® model with two injectors per detector. This is ideal for flash type luminescence assays as it combines high throughput with high sensitivity luminescence detection, therefore providing all tools for studying calcium related GPCR and ion channel assays. With MicroBeta® the GPCR assay can be run in single mode to determine agonist or antagonist effect of a molecule, or in dual screen mode to determine both agonist and antagonist response for one sample plate. In dual screen mode the plate is run successively, generating approximately 20 effective time points in a 384-well plate for a possible antagonist to interact with cell membrane targets.