FDSS7000EX FDSS/µCELL

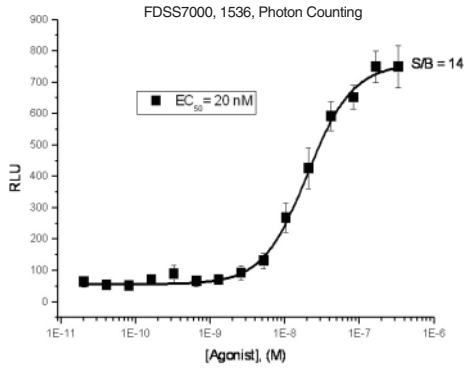
β-arrestin recruitment for GPCR Screening

Introduction

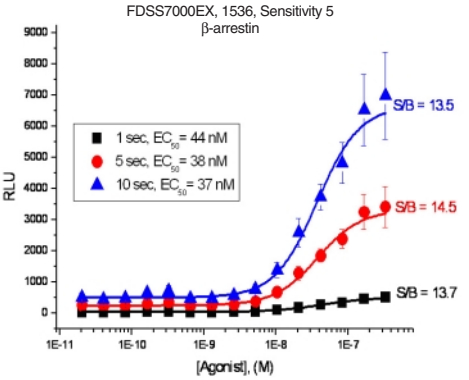
Over the years, it has been demonstrated that signaling through GPCR is not restricted to the type of G protein they are linked to. Downstream events can also be triggered by β-arrestin, a protein involved in signal regulation and receptor internalization. In order to detect β-arrestin recruitment by GPCR activation, DiscoveRx has developed the PathHunter luminescent b-arrestin assay. The latest version of this assay is the PathHunter eXpress β-arrestin protocol which was tested on Hamamatsu FDSS instruments (7000 and 7000EX) and the new FDSS/µCELL. The FDSS/µCELL (a compact, cost effective and simple system for MTS and assay development) and FDSS7000EX are both equipped with Hamamatsu's new EM-CCD camera (Electron Multiplied) for fluorescence and luminescence assays and the FDSS7000 is equipped with our trademark photon counting camera. In this context, we compared FDSS7000 and FDSS7000EX in 1536 format using a proven and reliable assay such as PathHunter. The same experiment was conducted in 384 well format using the FDSS/µCELL. This study shows the comparison (assay window, EC_{50} and signal to noise ratio) between the two types of detection systems using the same assay.

Materials and Methods

One day before the experiment cells were plated in 1536 well clear bottom plates (500 cells/well in 2 µl of culture medium) or in 384 well plate (5000 cells per well in 50 µl). The day of the experiment, 1 µl of compound (3x concentrated diluted in HBSS/20 mM Hepes/0.2 %/fatty acid free BSA) or 5 µl in 384 well format (5x concentrated diluted in HBSS/20 mM Hepes/0.2 %/fatty acid free BSA) were added in each well and plates were incubated 90 min at 37 °C. β-arrestin recruitment is detected following the addition of 1 µl in 1536 (or 12.5 µl in 384 well) of DiscoveRx PathHunter Glow reagent with a multidrop and incubation for 60 min at room temperature. The plates were read on FDSS7000, FDSS7000EX (both in 1536 format) and FDSS/µCELL (in 384 format) with exposure times of 1 s, 5 s and 10 s.

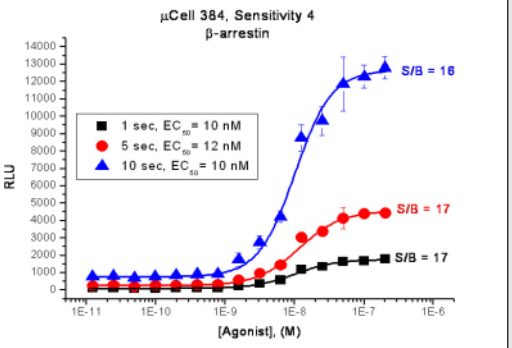


Reading time (s)	1	10
EC50 (nM)	20	20
S/N	17	14



Reading time (s)	1	10
EC50 (nM)	44	37
S/N	14	13.7

Fig. 1: Curve for PathHunter eXpress β-arrestin GPCR targets run on Hamamatsu FDSS7000 (left) and FDSS7000EX (right) with exposure times of 1, and 10 s



Reading time (s)	1	10
EC_{50} (nM)	10	10
S/N	17	16

Fig. 2: Dose response curve for PathHunter eXpress β-arrestin GPCR targets run on Hamamatsu FDSS/mCELL with exposure times of 1 s, 5 s and 10 s.

Results

In this study, we compared the performance of FDSS7000 (photon counting camera) with FDSS7000EX in 1536 format and FDSS/µCELL in 384 format (EM-CCD camera) monitoring the luminescence signal following β-arrestin recruitment. To obtain comparable results, maximum sensitivity was used on the FDSS7000EX, whereas sensitivity 7 (maximum 10) was used on the FDSS7000. Assay window is improved when the assay is run on the FDSS7000EX. No significant difference was observed in EC_{50} and S/N ratio between the two FDSS7000 systems. The 1536 format is not implemented on the FDSS/µCELL (made for MTS and assay development) so experiments were conducted in 384. Taking into account the difference in assay format, we found no significant difference between results obtained with the FDSS/µCELL and the FDSS7000.

Summary

The aim of our study was to compare two different detection methods for luminescence: the FDSS7000 using a photon counting camera, the FDSS7000EX and the FDSS/µCELL, both using an EM-CCD camera for fluorescence and luminescence. We observed no significant difference between the two detection methods and even noticed an improved assay window with the EM-CCD camera. The data shows that even though EM-CCD sensitivity is technically lower than photon counting, luminescent assays such as PathHunter can be successfully run on all our platforms.

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Cat. No. SBIS0082E02
NOV/2013 IP
Created in Japan (PDF)