slas 2012
1st ANNUAL CONFERENCE & EXHIBITION

FDSS/µCELL implementation for medium-throughput calcium assays

Jans M.*, De Raeymaeker M.*, Wesse A-S.*, Hens S.*, Van Rompaey L.*, Conrath K.* and Christophe T.*
*Galapagos NV, Generaal de Wittelaan L11 A3, 2800 Mechelen, Belgium
E-mail: rd@glpg.com

Introduction

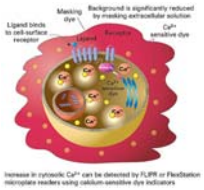
At Galapagos, a clinical stage biotech company developing novel mode-of-action medicines, a large number of novel targets is entering drug discovery. It is critical to find a convenient, fast and cost-effective solution for assay development and compound screening, particularly for GPCRs.

Objective: implementation of the FDSS/µCELL (Hamamatsu) as a medium throughput imaging plate reader for GPCR calcium flux assays at Galapagos, optimization of the settings of the FDSS/µCELL and extensive validation in comparison with the Flexstation 3 (Molecular Devices).

Methods

GPCR antagonist calcium flux assays were evaluated and optimized for batch processing on the FDSS/µCELL using the following protocol:

- Cell seeding (384 plate, 24h., 37°C)
- Loading with Ca^{2+} indicator (1h., 37°C)
- Offline addition of compounds to test (15min., 37°C)
- Injection by the reader of agonist at EC_{80} and simultaneous recording response (3min. on FDSS/µCELL versus 35min. on Flexstation 3)



Reference
http://www.moleculardevices.com/Products/Assay-Kits/GPCRs/FLIPR-Calcium.html

Optimization of settings of the FDSS/µCELL

	1	2	3	4	5
Volume (µl)	10	10	10	10	10
Aspirate speed [µl/s]	30	30	30	30	30
Aspirate height [mm]	1	1	1	1	1
Source plate trituration	5	5	5	5	5
Dispense speed [µl/s]	5	5	10	10	20
Dispense height [mm]	3	3	5	3	3
Destination plate trituration	0	5	0	0	0

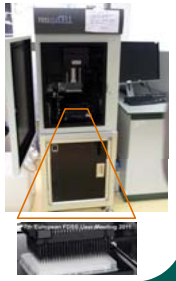
Figure 1. Optimization of dispensing parameters on FDSS/µCELL

10µl/s dispense speed with 3mm dispensing height allowed correct dispensing of the agonist EC_{80}

FDSS/µCELL (Hamamatsu)

+imaging plate reader
+filter optics
+384-channel pipettor
+imaging based detection of entire plate
+multiple compound/ligand dispensing
+active wash station and wipe function
+reading time: < 3min (384 wells)

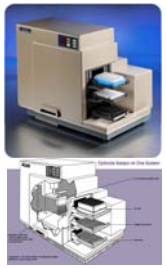
−no temperature control



Flexstation 3 (Molecular Devices)

+bench top multimode reader
+96-384 plate format flexibility
+temperature controlled

−low throughput (reading time > 35 min)
−no active wash station, tips not reusable
−column pipetting (16 channel pipettor) with regular crashes



Batch processing of plates on the FDSS/µCELL

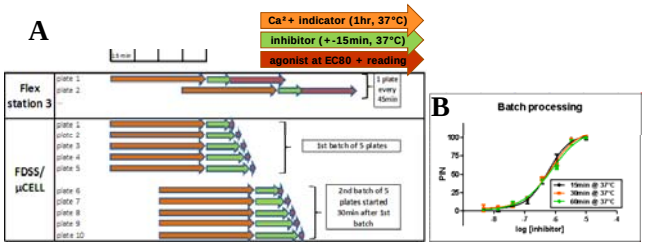


Figure 6. During batch processing, compounds to test are staying on cells for time varying from 15 to 60 min before the plates are read. IC_{50} and assay stabilities had to be evaluated. (A) Batch processing of plates on the FDSS/µCELL versus plate per plate processing on the Flexstation 3. (B) Effect of compounds incubation on compounds potency.

Throughput of > 8000 points per day ~ 10 times faster than Flexstation 3

Validation of the FDSS/µCELL in comparison with the Flexstation 3

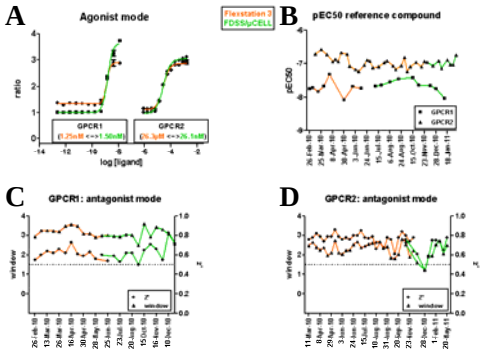


Figure 2. Comparison of 2 GPCR assays (named GPCR1 and GPCR2) on the FDSS/µCELL and the Flexstation 3 in agonist mode (A) and in antagonist mode: EC_{50} of the reference compounds (B), Z' and assay window (C&D)

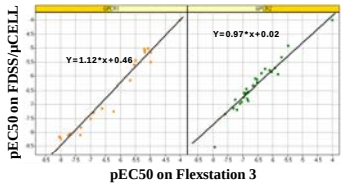


Figure 3. Comparison of inhibitors potencies obtained on FDSS/µCELL and Flexstation 3 for 2 GPCRs

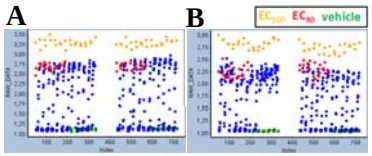
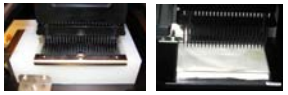


Figure 4. Robustness of assay parameters on Flexstation 3 (A) in comparison with FDSS/µCELL (B)

Antagonist Ca^{2+} assays validated on FDSS/µCELL
Similar assay quality between FDSS/µCELL and Flexstation 3
Good correlation between inhibitors potency on FDSS/µCELL and Flexstation 3

Active wash station and wipe function



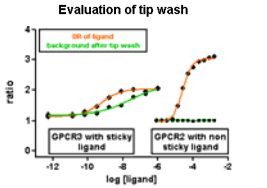


Figure 5. Agonist ligands (sticky and non sticky) injection by FDSS/µCELL and plate reading for 2 GPCRs (named GPCR2 and GPCR3). Tips are then washed with the active washing station followed by vehicle injection and plate reading.

Repeated use of tips feasible for non sticky ligands

Conclusions

It is demonstrated that the FDSS/µCELL was successfully implemented for medium throughput GPCR antagonist calcium assays:

- 10µl/s dispense speed with 3mm dispense height allowed correct dispensing of the agonist EC_{80} in the cell plate
- Efficient tip washing possible (not for sticky ligand)
- Z' >0.5, good reproducibility of EC_{50} of reference compounds
- Potency of inhibitors comparable between Flexstation 3 and FDSS/µCELL
- Running sets of 5 plates showed stable assay window on the FDSS/µCELL allowing a throughput of more than 8000 points per day (~10 times faster than the Flexstation 3)

Galápagos

Poster available online at:
www.glpg.com