Miniaturization of A Calcium Mobilization Assay Using 384 Well Low Volume Microplate

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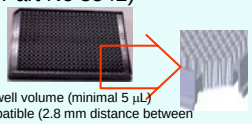
Introduction

Intracellular calcium mobilization is frequently used to investigate GPCR-ligand interactions and to screen for compounds that interfere with such interactions. Currently, this assay is predominantly performed in 384 well format with total volume ranging from 80 µL to 100 µL. In this poster, we will show a calcium mobilization assay that is miniaturized to 25-40 µL using the new Corning® 384 well low volume (LV) microplate. The results show that the data quality and assay performance on this LV microplate are comparable to those obtained from 384 well normal volume (NV) plates and better compared to a competitor LV microplate. We also present data that demonstrate the impact cell culture conditions as well as plate readers on the performance of this miniaturized assay.

Materials & Methods

❖ 384 well low volume (LV) microplate: (Corning® Part No 3542)



- 50 µL total well volume (minimal 5 µL)
- FLIPR compatible (2.8 mm distance between well bottom and plate bottom)

❖ Cell line used:

M1WT2 (ATCC, CRL-1984): CHO-K1 cells transfected rat M1muscarinic acetylcholine receptor. Expression level is ca. 370 fmole of receptor protein per mg of membrane protein.

❖ Calcium Mobilization Assay:

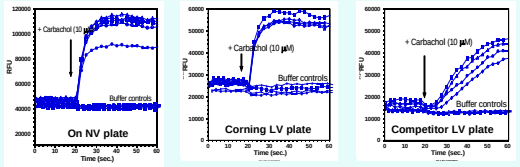
-- Calcium 3 Assay kit from Molecular Devices, inc.

- ❖ Lift M1WT2 cells from culture flask using a non-enzymatic dissociation solution.
- ❖ Seed cells at 5000 cells/well (unless specified in graphs) in 10 µL volume (HAMs F-12 + 2%FBS + 10 µg/µL g418);
- ❖ Allow the cultures to growth overnight (unless specified) in humidity controlled CO_2 incubator;
- ❖ Add equal volume of calcium dye solution;
- ❖ Let incubate at 37°C for 30 min, then at RT for 30 min;
- ❖ Read the signals on Flexstation® (Molecular Devices, Inc.)

Results

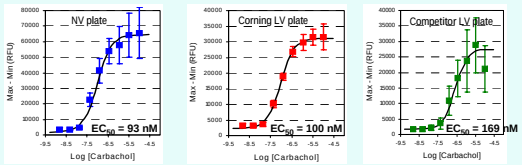
I. Assay Performance Comparison:

A. Time dependent kinetics:



- Rapid Calcium mobilization upon the addition of carbachol was obtained from Corning LV microplate and the kinetics is very similar to that from NV microplate. n=5.
- Overall signal strength and background fluorescence on LV plates were reduced compared to that from NV plate.
- Calcium response on competitor LV plate was delayed and the kinetics is much slower compared to NV microplates.

B. Dose response curve – EC_{50} comparison



- Similar dose response curve and EC_{50} to carbachol stimulation were obtained from both Corning LV and NV plate. Although assay window from LV microplates were narrowed, less data variation was seen on Corning LV microplates. n=5.
- The competitor LV microplates showed much higher data variation compared to Corning LV microplates.

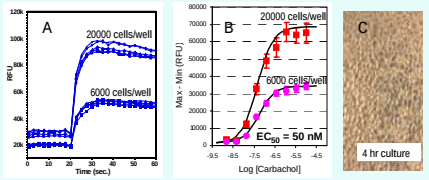
C. Across plate variations (n=92) :

	S:B ratio	Z'
NV plates	26.31	0.44
Corning LV	19.5	0.51
Competitor LV	20.08	0.37

- Signal window is smaller on LV plate than that on NV plate;
- Overall plate performance on Corning LV is better than those on NV plate and competitor LV plates.

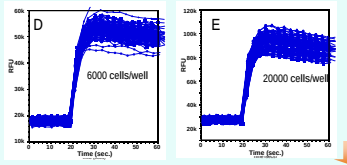
II. Impact of cell culture conditions on assay performance:

The graphs below were generated using 3-4 hr old cultures (3-4 hours after seeding in microplates).



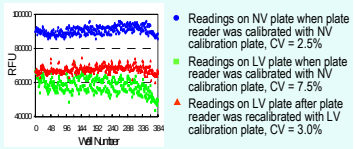
F	S:B	CV	Z'
3-4 hr cultures (6Kcells/well):			
Plate 1	36.30	6.89	0.77
Plate 2	33.82	7.10	0.71
Plate 3	32.04	6.50	0.75
Over night cultures (6kcells/well):			
Plate 4	19.50	14.00	0.51
Plate 5	20.05	10.96	0.61
Plate 6	18.02	11.63	0.58
3-4 hr cultures (20kcells/well):			
Plate 7	55.33	6.42	0.71
Plate 8	46.90	7.38	0.76
Plate 9	49.36	7.47	0.75

- At the same seeding density (6000 cells/well), 3-4 hr cultures yielded similar signals (A) and slightly lower EC_{50} (B) compared to overnight cultures;
- Assay signal window obtained from 3-4 hr cultures was 75% greater than that from overnight cultures with the same seeding density (see Table F);



- Assay signal window can be further improved (1.5 times more) with 3-4 hr cultures when seeding at higher seeding density (E & F);
- Intra-plate and inter-plate variations with 3-4 hr cultures were much smaller than those from overnight cultures (D, E & F);
- Overall microplate performance (Z') with 3-4 hr cultures was much more improved comparing to overnight cultures (see Table F).

III. Plate reader consideration in assay miniaturization:



LV microplates provide a smaller well bottom area compared to NV plates. Thus, the proper alignment of the optical head on PMT plate readers with wells on LV plates is more critical. The graph above shows that when a PMT plate reader generates acceptable across plate CVs for NV plates, it does not necessary mean that the optical alignment is optimal for LV plates. It is critical that the plate readers are calibrated with LV calibration plates before performing assays on LV plates.

Conclusions

- ► Calcium mobilization assays can be miniaturized in 384 well format using 384 well low volume microplate;
- ► Corning® 384 well LV microplate performed better than competitor LV microplate.
- ► The miniaturized assay reduce reagent cost by >50%;
- ► Reduced cell culture period can significantly improve the assay performance through 1) increased assay signal window and 2) reduced intra-plate and inter-plate variations;
- ► More precise alignment for PMT plate readers is needed to obtain better quality data.