

Intracellular Calcium Assay on NOVOstar Microplate Reader

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Application Note 104

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- Automated fluorimetric calcium assay
- Fast screening of compounds in 96 or 384 format
- Dose response assay

Introduction

Many biological signals are transmitted by increasing or decreasing the intracellular calcium concentration (receptors, ion channels). Intracellular calcium assays are therefore used for studying a great number of biological systems. Such assays have often been done using microplate readers. Here cells are typically grown in wells and the sample/compound is added from a container using a fixed pump/injector or manually.

To test the next sample the system has to be rinsed and the sample container replaced manually. This makes this type of assays slow and labour intensive.

This application note describes an intracellular calcium assay done using a novel microplate reader, NOVOstar with integrated pipetting system from BMG LABTECH. The NOVOstar is capable of transferring samples from one microplate to another (e.g. from a plate containing samples to a plate containing cells), greatly increasing the throughput of samples.

Materials

All materials were obtained through normal distribution channels from the manufacturer stated.

- Cells: CHO cells stably expressing a calcium channel.
- NOVOstar, BMG LABTECH, Offenburg, Germany.
- FLIPR[®] calcium assay kit, Molecular Devices Corporation, Sunnyvale, Ca.
- Viewplate[™] -96, Black, Packard #6005182, Groningen, The Netherlands.
- TC-384 well plate µclear, Greiner #GR-781091, Frickenhausen, Germany.

Standard medium and buffers are used. In addition, consumables such as pipette tips and tubes were used as needed from various manufacturers.

Experimental

The following is a brief description of the dye loading of cells:

The day before the assay cells are seeded in 96-well plates at 16000 cells/well (100 µL) or in 384 well plates at 4000 cells/well (25 µL). Cells are grown over night in a humidified incubator at 37°C and 5% CO₂.

At the day of the experiment the cells are removed from the incubator and loading buffer from the FLIPR[®] calcium assay kit is added (25 µL

for 384 well plates and 100 µL for 96 well plates). The cells are then incubated for 1h at 37°C.

You can also use more common calcium indicators like Fura-2, Fluo-4 and Calcium-Green[™]. A more detailed description of the cell dye loading procedure is included in the assay kit.

Protocol

The loaded cells are placed in the measurement position in the NOVOstar, and the sample plate is placed in the reagent plate position. The assay is done using the following parameters:

Excitation filter is 485 nm, emission filter is 520 nm, injection speed 100 µL/sec, dispense depth and aspirate depth are in accordance with the volume in the well and gain is 1800.

Before sample injection 3 measurements (one per second) are done to establish the baseline (kinetic window 1), then sample (4 µL for a 96-well plate, and 1 µL for a 384-well plate) is transferred from sample plate to cell plate. It takes 5.5 s for the system to transfer the sample, so the sample injection is started after 8.5 (3+5.5) seconds. At this point the sample is injected and measurements are resumed simultaneously (one per second) for 40 seconds (kinetic window 2). Immediately after sample injection mixing is performed by the system (50 µL is pipetted up and down 3 times in the well for a 96-well plate, 17 µL for a 384-well plate).

Directly after mixing the pipettor needle is moved to the wash station and the needle is washed with 0.1 M NaOH followed by rinsing with system fluid (50% ethanol). The procedure described above is then repeated for the rest of the wells.

Results and Discussion

Screening Assay

A 96-well plate with cells loaded with calcium responsive dye was placed in the measurement plate position in the NOVOstar, and a reagent plate containing samples/compounds was placed in the reagent plate position. The assay was then done as described above.

The curves in figure 1 and the base line corrected values in figure 2 show clearly that 4 of the samples are agonists.

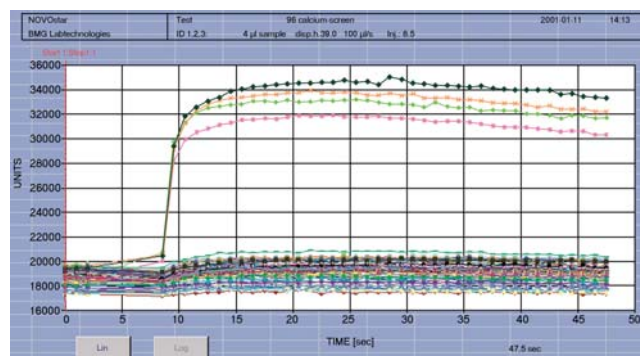


Fig. 1: Calcium response curves of 96 samples added to wells containing cells.

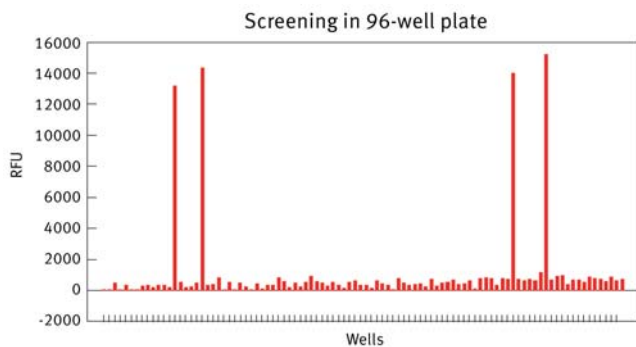


Fig. 2: Specific response: Baseline corrected response after 25 seconds. Measurement is done in a 96-well plate.

A 384-well plate with cells loaded with calcium responsive dye was placed in the measurement plate position in the NOVOstar.

To compare the assay with the 96-well assay above, the same 96 samples were then plated out in 96 wells in a 384-well plate, and the plate placed in the reagent plate position. The assay was done as described above and the baseline corrected values are shown in figure 3.

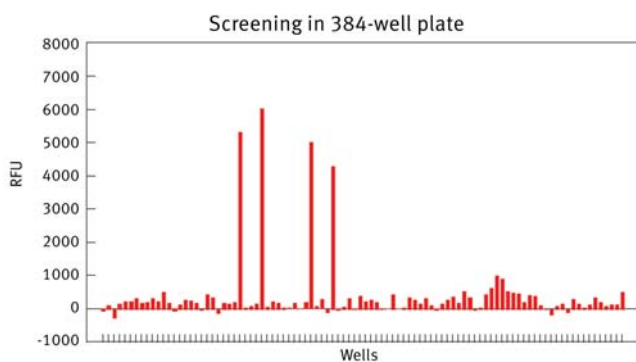


Fig. 3: Specific response: Baseline corrected response after 25 seconds. Measurement is done in a 384-well plate.

The results obtained using 384-well plates are very similar to the results obtained using 96-well plates. Small differences may be due to differences in plate optics and in cell growth.

Dose Response Assay

A 96-well plate with cells loaded with calcium responsive dye was placed in the measurement plate position in the NOVOstar. A dilution series with 7 dilutions was made with one of the active compounds

found above. This was then plated out in a 96-well plate, one well per dilution, and the plate placed in the reagent plate position. The kinetics of the raw data curves is shown in figure 4 and the baseline corrected response is shown below in figure 5.

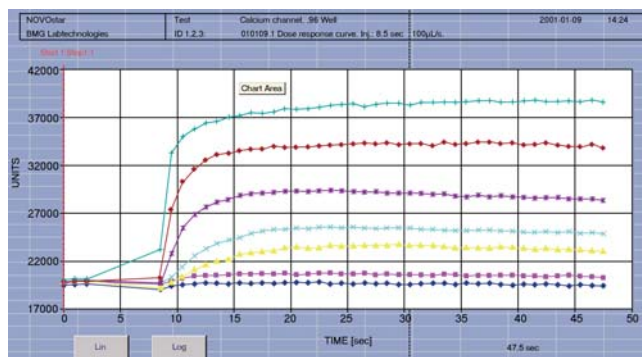


Fig. 4: Compound at 7 different concentrations were added to wells containing cells.

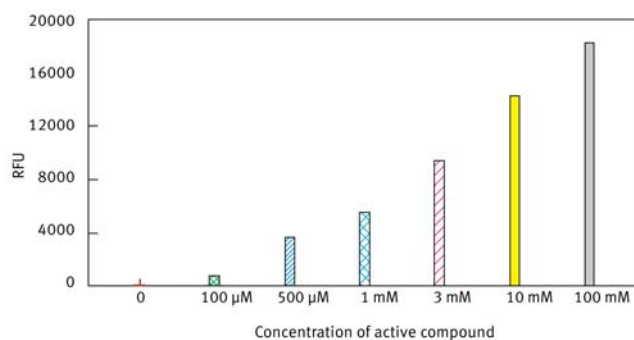


Fig. 5: Specific response: Baseline corrected response after 25 seconds for 7 different agonist concentrations.

Conclusion

Using the NOVOstar we have successfully screened 96 compounds in less than 90 minutes with minimal „hands on“. This is virtually impossible to do using conventional microplate readers. If it were to be done it would take days and would require constant attention by technical staff. The screening can be done in both 96- and 384-well plates with similar results. We have also successfully used the NOVOstar for generating dose response curves. This is another assay type that can be done with great advantage in the NOVOstar due to the robotic capability of the instrument.

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