

# Cell-based Assays using EnVision™ Multilabel Plate Reader with Dispenser



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## INTRODUCTION

The EnVision™ multilabel plate reader has been equipped with dispense options to meet the needs of cell-based drug discovery assays. Detection of GPCR-mediated calcium signaling, or ion channel activation, where signal levels peak in seconds and decay in tens of seconds require measurement of the emitted light to be monitored kinetically at the same time as dispensing.

The present study shows the ability to perform calcium flux assays with fast kinetics using EnVision™. Functional cell-based GPCR assays with both adherent - and suspension cells using the calcium-sensitive dyes Fluo-4 AM and Fura-2 AM were measured using EnVision™ equipped with dispensers. Ratiometric VSP assays on EnVision offers system for measuring ion channel modulators.

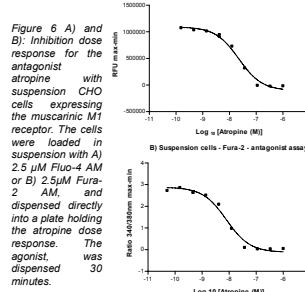
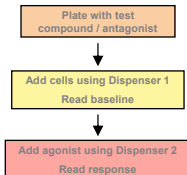
## INSTRUMENT FEATURES

2103 EnVision™ Multilabel Plate Readers features modular label-specific optical mirror modules, high energy flash lamps, and high speed detectors. The instrument is designed to provide the greatest configuration flexibility possible, including accepting micro plates from 6 to 1536 wells. EnVision™ can handle kinetic measurements for enzyme assays and scanning of the well area for cellular assays. Focus point adjustment can be done with the Measurement Wizard to the bottom of the well or into the liquid to reach the maximum signal. The dispenser unit is equipped with two pumps. The dispense can be done in 96- or 384-well format with volumes ranging between 2- and 475 µL. The dispense speed can be adjusted from gentle for cellular assays to fast, and it can be done in real-time, or it can be a pre- or post-measurement dispense. The two tips can be set to dispense simultaneously to maximize the throughput.

## CALCIUM ASSAYS WITH CELLS IN SUSPENSION

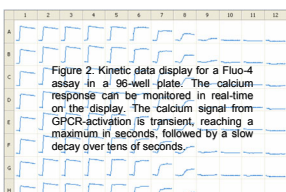
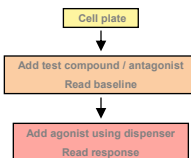
In order to increase the throughput for the calcium measurements it was preferable to be able to dispense cells instead of compounds. EnVision™ was capable of dispensing cells without affecting the calcium activation in the cells.

Figure 5. Assay flow chart for antagonist calcium assays with cells in suspension.



## CALCIUM ASSAYS WITH ADHERENT CELLS

Figure 1. Assay Flow chart for calcium assays with adherent cells.



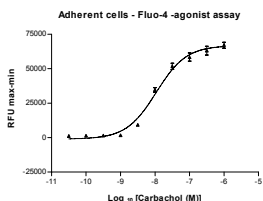
Instrument protocol for kinetic calcium measurements. The baseline was read for 5 seconds prior to agonist addition, after which the measurement was continued for up to 1 minute. The agonist was dispensed in a volume of 50 µL, at a speed of 200 µL/second.

CHO cells transfected with the muscarinic cholinergic receptor M1 were plated at a density of 40000 cells/well in white, clear bottom, 96 well TC-plates (Costar) and grown for 18 – 20 hours. The cells were loaded with 2.5 µM Fluo-4 AM or Fura-2 AM dyes for 45 minutes at 37° C.

For antagonist assays, 50 µL of 3x opto dispense was added and the cells were incubated at 37° C for 30 minutes prior to the measurement. The agonist was dispensed on-line using the EnVision™ dispense unit.

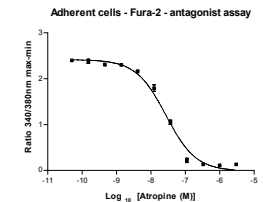
The data from Fluo-4-assays was expressed as the maximum RFU value per well minus the minimum value (max-min) after agonist addition.

Figure 3: Calcium dose response in CHO cells expressing the muscarinic M1 receptor subtype. The cells were loaded with 2.5 µM Fluo-4 AM and stimulated with increasing concentrations of carbachol. Data from a representative experiment are shown as mean +/- SD from 8 wells.



Using the ratiometric dye Fura-2 AM, the artifacts from variances in cell number and dye uptake was eliminated. The instrument was set up to excite at two wavelengths 340 – and 380 nm while the emission was recorded at 510 nm. The data from the dual excitation measurements was expressed as the maximum ratio per well minus the minimum ratio (max-min) after agonist addition.

Figure 4: Inhibition dose response for the antagonist atropine in CHO cells expressing the muscarinic M1 receptor. The cells were loaded with 2.5 µM Fura-2 AM, and stimulated with 1 µM carbachol.



## HTS CALCIUM ASSAYS

When the kinetics of the calcium response is known, the measurement time can be decreased to two measurement points per well, by only reading one point on the baseline and one point on the response peak, or - plateau. EnVision™ has the capability for handling sample wells in the form of operation blocks, or groups. A high throughput assay alternative to the kinetic measurement was developed, where the samples were measured using the operation block option. Using this method, the read-time for one 96-well plate was reduced eight-fold compared to measurements with standard plate readers.

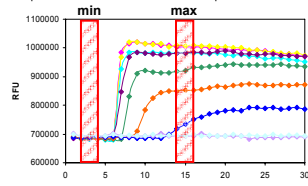
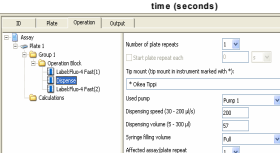


Figure 7. Typical kinetic calcium response for a Fluo-4 assay. The cells were stimulated with different concentrations of agonist.



Procedure: The operation block option was chosen in the software. The baseline for the wells within the operation block were first read, then the agonist was dispensed into all these wells, after which the first well was measured for plateau value after agonist dispense. 8 wells per operation block was calculated to be the suitable amount of samples for this particular calcium response. When the dispense volume was 57 µL, the total volume in the syringe was adequate for 8 wells, so the refilling of the dispenser was done between operation blocks. The second read was done at a speed of 250 flashes per well to keep the same pace as the dispense. In this way, all samples were read at exactly the same time after the agonist dispense.

## Adherent cells - Fluo-4 - antagonist assay

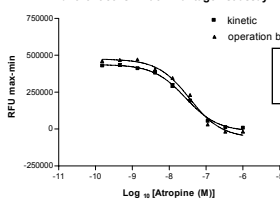
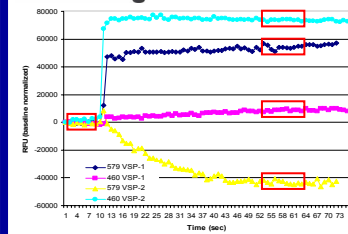


Figure 8: Comparison of the kinetic and the fast mode. Dose response for the antagonist atropine in Fluo-4-loaded cells. The CV% for a 96-well plate was 5.4% when measuring kinetically, and it was 4.7 % when using the fast measurement mode (data not shown).

## VSP ION CHANNEL ASSAYS

Ion channels are critical for generating and controlling electrochemical gradients across biological membranes. Ion flux through any ion channel will cause a change in the electrochemical gradient and result in a change in the cell membrane potential. The FRET-based Voltage Sensor probes from Invitrogen™, provides a fluorescent assay technology for measuring changes in cellular membrane electrical potential. The technology is based on energy transfer between a mobile, voltage-sensitive acceptor oxonol and a membrane-bound coumarin phospholipid donor.

EnVision™ was set up to excite the samples at 400 nm and read the emission at two different wavelengths. For the donor, a coumarin lipid - CC2-DMPE, emission was recorded at 460 nm. The acceptor is a highly fluorescent, negatively charged hydrophobic ion for which a 579 nm emission filter was used.



HEK293 cells over-expressing the muscarinic M3 receptor were plated at a density of 30000 cells / well in 96-well Poly-D-Lysine treated plates (Greiner) and grow overnight. The cells were loaded with 6 µM CC2-DMPE for 30 minutes followed by loading with DISBAC(3) for 30 minutes prior to measuring depolarization using EnVision™. The baseline was read for 10 seconds prior to dispensing VSP-1 or VSP-2 (high KCl) buffers using EnVision™ and monitoring the depolarization response.

Figure 9. Averages were calculated for polarized (pre-dispense) and depolarized (post-dispense) values. The measured values were background corrected using measurements from wells without cells. The ratio was then calculated:

$$\text{Response Ratio} = \frac{460 \text{ depolarized} / 579 \text{ depolarized}}{460 \text{ polarized} / 579 \text{ polarized}}$$

		Response Ratios					
Plate		VSP-1	AVG	SD	VSP-2 (High KCl)	AVG	SD
1		0.94	0.76	0.85	0.79	0.86	0.84
2		0.81	0.76	0.87	0.76	0.86	0.81
1		3.25	2.33	2.59	3.27	2.83	2.85
2		2.57	3.36	2.84	2.65	2.27	2.74

Figure 10. Typical ratios obtained with HEK293 cells in a VSP assay dispensing high KCl buffer for depolarization of the cells.



## CONCLUSIONS

EnVision™, equipped with dispensers, was suitable for functional cell-based GPCR assays using calcium-sensitive dyes for measurements of agonist- and antagonist assays.

EnVision™ was capable of dispensing cells without affecting the calcium response in the cells. The throughput was increased significantly for calcium assays where the measurement time could be decreased to two reads per well when the plate was divided into groups.

EnVision™ was also able to read ratiometric assays, both Fura-2 calcium- and ion channel (VSP) assays.