



Advantages of built-in dispenser of Varioskan™ spectrophotometer and spectrofluorometer

This paper describes the benefits of using the built-in dispenser of Thermo's Varioskan spectrophotometer and spectrofluorometer.

Approximately 50 % better reagent addition precision and 5 - 10 % better accuracy is obtained with the automatic dispenser compared to manual pipetting.

The automatic dispenser is also shown to be a necessity for fast kinetic reactions, such as the measurement of Ca²⁺ with Fluo-3 fluorochrome.

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Abstract

Varioskan spectrophotometer and spectrofluorometer has a unique feature of an optional built-in dispenser. The benefits of the dispenser are described in this note together with some examples about efficient use of the dispenser to optimize assay performance. The given examples include both photometric and fluorometric measurement, including fast kinetic fluorescence measurement with Fluo-3 label used for Ca²⁺ binding applications.

Improved precision and accuracy

An automatic dispenser offers improved volume accuracy and precision over the manual addition

of liquid. This is shown with red photometric dye (Ponceau S) in water. Two different volumes, 5µl and 50µl, were added to the wells of 384-well microplate manually and with the Varioskan dispenser. The wells were then filled to 100 µl with Multidrop 384 dispenser and absorbance was measured at 540nm. The absolute volume of the liquid was measured by weight. The results are shown in Figures 1 and 2. Based on these experiments one can easily conclude that the built-in dispenser on the Varioskan offers better accuracy and precision than manual reagent addition, especially with small volumes. The auto-matic dispenser improves the precision of the reagent addition.

Essential for fast fluorometric Calcium assays

When intracellular Ca^{2+} is measured with fluorometry the common procedure is as follows: Cells are loaded with fluorometric label, a sample activating Calcium channels is added with the automatic dispenser and changes in intracellular Ca^{2+} levels are followed based on the changes in the fluorescence using a kinetic measurement¹⁻⁴. In this assay it is essential to optimize the sample dispensing speed so that the kinetic fluorescence curve is based on the activity of the Calcium channels, not on the reagent addition settings. This principle is shown in Figure 3. A green line shows theoretical response of the Ca^{2+} channel, blue line shows the formation of fluorescence signal in cell free system with high reagent addition speed and red line is a signal response with slow reagent addition speed. As seen, if the sample/reagent dispensing speed is not correctly selected, the resulting kinetic curve will not show the cellular response correctly.

The automatic reagent dispensing with adjustable dispenser settings gives special possibility to optimize the sample/reagent addition parameters in fast fluorometric reactions. This feature is demonstrated with the Ca^{2+} binding of Fluo-3 fluorometric label (Molecular Probes Inc., Eugene, OR). Fluo-3 is almost non-fluorescent without Ca^{2+} present in the solution and becomes highly fluorescent when bound to Calcium. Therefore, it is a common fluorochrome for the detection of the Calcium channel activity¹⁻⁴.

First, 100 μl of Ca^{2+} solution was added with different reagent dispensing speed settings into 100 μl of Fluo-3 solution to reach satura-

tion concentration for the binding (40 μM). The kinetic fluorescence curves were measured at 506 nm excitation with 5 nm bandwidth and 526 nm emission with 12 nm bandwidth. The results from the tests are shown in Figure 4. From these curves one can determine the time how long the dispensing action takes with each speed setting. It is known that Ca^{2+} /Fluo-3 binding is an extremely fast reaction³, so dispensing speed that produces full fluorescence signal in less than 0.15 seconds (dispenser speed setting 18) was selected for the further tests. This produces a full signal in short enough time frame. For example, with dispensing speed setting 14 it takes about 0.4 seconds to reach the full signal and therefore it is not useful for rapid assays. On the other hand, both the dispensing precision and accuracy are better with lower speed settings, so the lowest possible speed giving adequate performance should always be selected.

Linearity of Ca^{2+} /Fluo-3 binding was tested by adding 100 μl of Fluo-3 solution into Ca^{2+} solutions with different concentrations (100 μl) with the selected dispenser speed setting (18). Kinetic fluorescence curves of this test are shown in Figure 5. Ca^{2+} calibration curves collected from the different time points are shown in Figure 6. Figures 5 and 6 clearly show that the selected dispensing speed is suitable for the assay. A constant signal is achieved after 0.15 seconds and the expected dose response of Ca^{2+} concentration is easily detected. As shown by Figure 6 there is no change in the calibration curve after 0.2 seconds but the calibration curves obtained before that time are clearly different from the others.

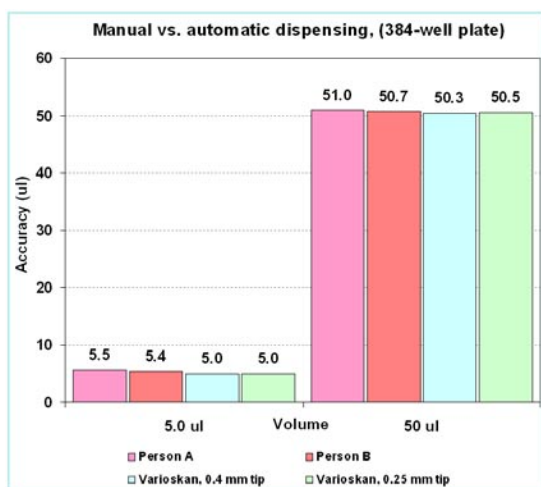


Figure 1. Comparison of accuracy between manual and automatic dispensing.

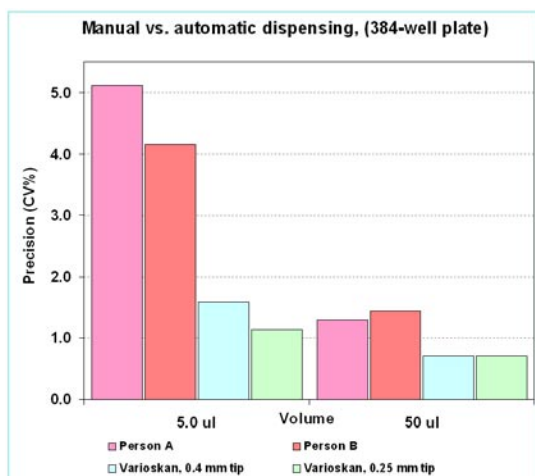


Figure 2. Comparison of precision between manual and automatic dispensing.

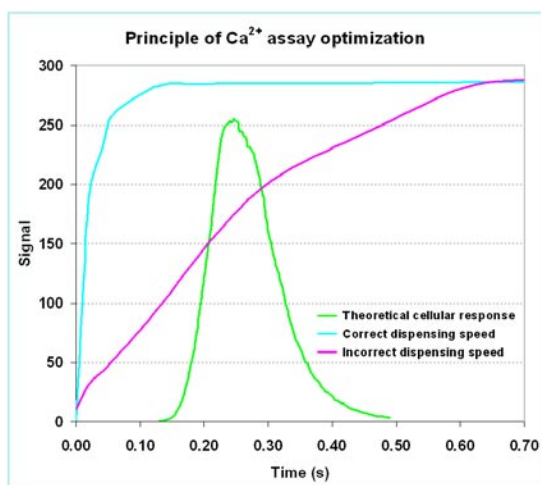


Figure 3. Theoretical principle of Ca^{2+} assay optimization.

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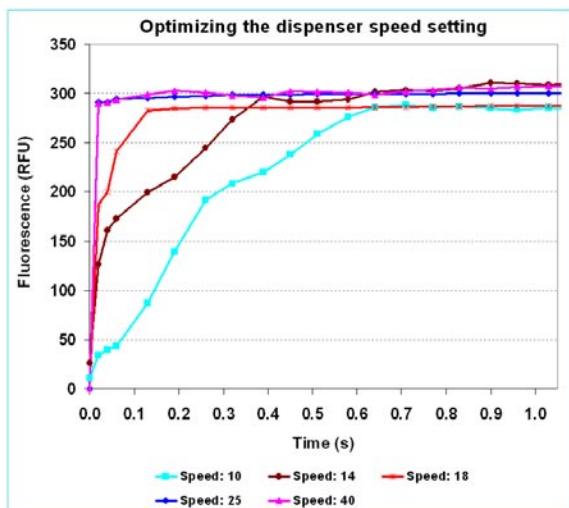


Figure 4. Kinetic fluorescence curves of Ca^{2+} /Fluo-3 complexes with different dispenser speed settings.

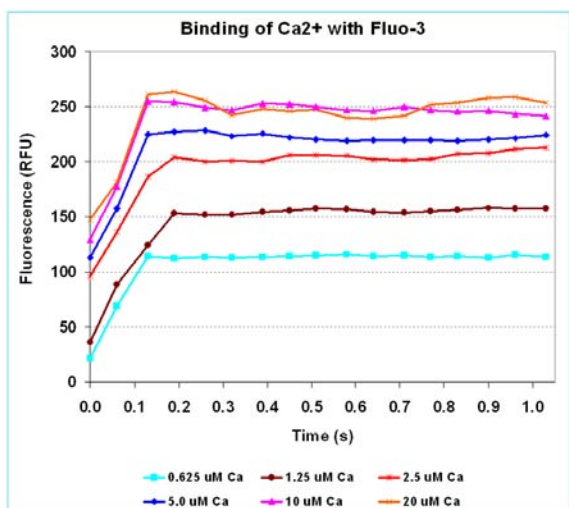


Figure 5. Kinetic fluorescence curves of Ca^{2+} /Fluo-3 complexes with different Ca^{2+} concentrations.

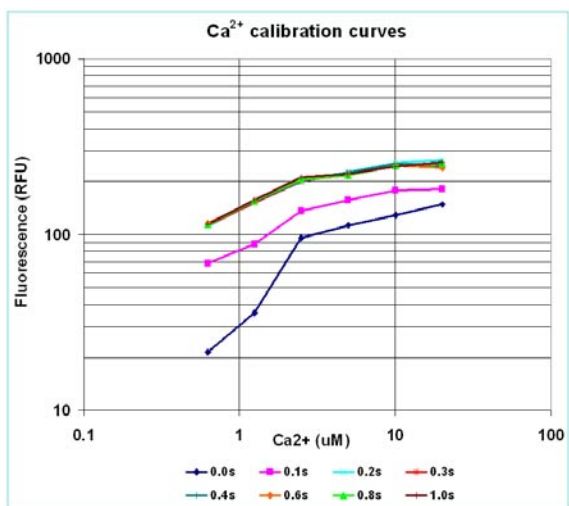


Figure 6. Calibration curves of Ca^{2+} /Fluo-3 complexes with different time points.

Varioskan with built-in dispenser is an ideal solution for the follow up of fast fluorometric reactions because the speed of reagent addition is easily optimized for each assay with automatic dispenser. This feature is especially useful in fast fluorometric Ca^{2+} assays where the automatic dispenser is an absolute requirement and the flexibility of dispenser settings give an extra advantage for the assay.

Improved operational safety

A built-in dispenser is especially advantageous when hazardous chemicals are used. When chemicals are added using the dispenser, the risk of reagent spilling and contamination is remarkably reduced. This feature is of particular importance in drug discovery where several compounds to be studied are either highly toxic or their chemical safety is unknown.

References

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