

Assay-ready Compound Dilution Using a mosquito[®] Low Volume Pipettor

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Abstract

Dilution is an integral step in the pharmacological profiling of compounds, in particular, to determine their concentration-related effects against therapeutics targets. Routinely, serial dilutions are generated in a series of tubes or adjacent wells in microplates requiring a secondary step of transferring the compound dilutions into the assay plate. mosquito[®] is a low volume liquid handling instrument combining the advantages of a disposable tip system with those of a positive displacement pipette.

The mosquito has been used to perform low volume serial dilutions in sitting drops located on the bottom of 96 and 384 well microplates. Typically the volumes were restricted to 1-2 microlitres which permitted use of the dilution plate as the assay plate. Results for a high content analysis are presented. Use of intra-well dilution can reduce compound usage and consumable costs, as well as increasing assay throughput.

Introduction

Determination of the dose-dependency of a compound's activity against a target is central to building the structure activity relationships upon which rational drug design depends. The compound dilutions required are routinely performed in dilution plates and then these are subsequently transferred into the final assay plate. The availability of low volume pipettors now enables alternative protocols to be adopted and these include intra-well dilution in volumes of 1 microlitre or lower. This has the benefit of reduced compound usage, plus the possibility of using the dilution plate as an assay plate.

Here we present data generated using mosquito for intra-well compound dilution in 96 and 384 well microplates. Dilutions were performed in sitting drops on the bottom of each well. Its application to high content screening has been demonstrated using the Acumen Explorer[™], a laser-scanning fluorescence microplate cytometer.

1 mosquito Instrument



mosquito is a low volume liquid handling instrument combining a disposable tip system with a positive displacement pipette. mosquito is capable of pipetting volumes from 1.2 µL down to 50 nL with no washing required.

2 Sitting Drop Serial Dilution

1. Add diluent
2. Add stock to first well



3. Serially dilute
4. Add cell suspension

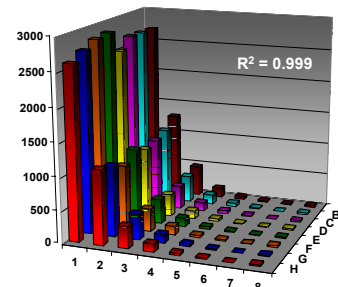


3 Sitting Drop Dilution on a PVC Sheet



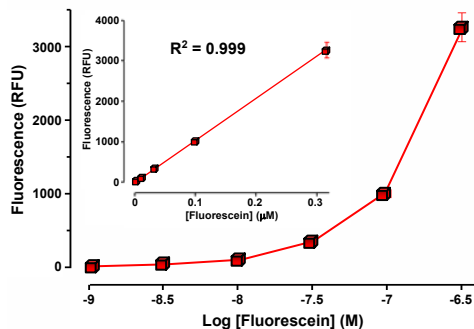
Rhodamine (1 mM) was diluted using PBS on a PVC sheet. Dilution volumes – 460 nL stock : 1,000 nL diluent.

4 Sitting Drop Dilution in 384 Well Plate



Fluorescein was diluted using PBS in a low volume 384 well plate (ShallowWell, Nunc) which has conical, flat-bottomed wells. Dilution volumes - 315 nL stock : 685 nL diluent.

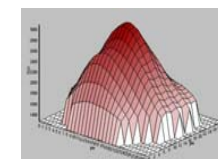
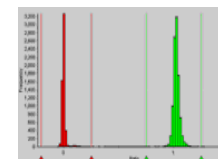
5 Sitting Drop Dilution in 96 Well Plate



Fluorescein was diluted using PBS in a 96 well plate (BD Falcon). Dilution volumes - 460 nL stock : 1,000 nL diluent.

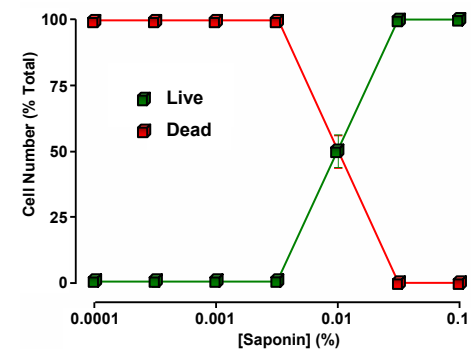
6 High Content Analysis in 96 Well Dilution Plate

Cytotoxic compounds were serially diluted using PBS in a 96 well plate (BD Falcon) to create 1 µL drops of 100X stocks. Jurkat cells were added to each well (100 µL) and the plate incubated for 1 hour @ 37°C / 5% CO₂. Cytotoxicity was determined using 1.0 µM calcein AM and 2.0 µM propidium iodide (Molecular Probes) fluorescent stains. The number of live and dead cells in each well was quantified using an Acumen Explorer laser scanning fluorescence microplate cytometer (TTP LabTech).



Classification of live (green) and dead (red) cells based on fluorescence colour ratio.

3D view of dead cell stained with propidium iodide.



Conclusion

We have shown that the mosquito is capable of accurately performing serial dilutions in sitting drops located on PVC sheets or within the wells of microplates. Use of total volumes of 1 – 2 µL allows use of the dilution plate as the assay plate for homogeneous and cell-based biochemical assays.

This conserves compound through use of small quantities of stock solution and the consumption of the entire compound dilution. Intra-well dilution can thus reduce compound usage and consumable costs, as well as increasing assay throughput.