automation and miniaturisation of compound serial dilution using the mosquito® HV

key benefits

- high accuracy
- high precision
- high throughput

introduction

Compound screening requires the serial dilution of lead compounds at all stages of development from early secondary screening to toxicity studies. Most of these assays are high throughput, using automation with minimal sample volumes to save time and money, but there are a number of limitations associated with doing this. Some of these include achieving high accuracy and precision, avoiding large dead volumes, ability to aspirate and mix, flexibility to use different plate formats and perform different experimental set-ups.

SPT Labtech's automated liquid handling range overcomes all of these issues. The mosquito HV (Fig 1) is extremely accurate within the 500 nL to 5 µL volume range across a wide range of liquid viscosities, in 96-, 384- and 1536-well plate formats. A serial dilution wizard in the mosquito software allows the user to very quickly and easily configure, generate and save a serial dilution protocol. The user can either choose from default protocols or define their dilution ratio, select the number of serial dilution points, the spacing of columns, the mixing cycle – and even opt to perform the serial dilution backwards. Standard protocols like 'assay ready' dilutions, mother/daughter plate replication, or plate reformatting are all very easy to perform on mosquito and do not require set-up changes to the instrument. This sort of process flexibility is essential to high throughput screening labs.

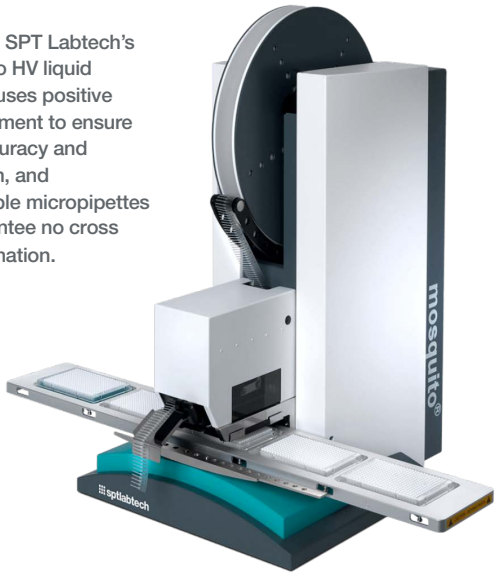

Figure 1. SPT Labtech's mosquito HV liquid handler uses positive displacement to ensure high accuracy and precision, and disposable micropipettes to guarantee no cross contamination.

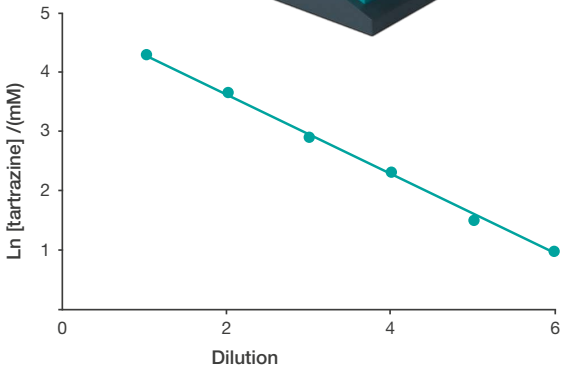

Figure 2. mosquito HV accuracy of pipetting a low volume 2-fold serial dilution of tartrazine (72 mM) using SPT Labtech's LVSD plate for the serial dilution.

case study 1:

High accuracy and precision with two 384-well plate types

method

The stock solution (tartrazine, 72 mM) was manually dispensed into the first column of a 384-well low volume V-bottom plate (Greiner Bio-One), followed by the diluent (DMSO) into the second column. This was repeated in 5 plates to measure mosquito's precision. Using the mosquito serial dilution wizard a two-fold dilution series was set up, mixing 5 times per dilution with a transfer of 600 nL of stock into each well containing 600 nL of DMSO. 500 nL of each dilution was stamped to a 384-well flat bottom plate (Greiner Bio-One) that had been previously loaded with 50 µL of buffer required for optimal measurement on the FLUOstar Optima plate reader (BMG Labtech). The absorbance was measured at 430 nm (+/- 5 nm) and the %CV was calculated. This was then repeated for SPT Labtech's Low Volume Serial Dilution (LVSD) plate type.

result

Accuracy of the serial dilution was determined by calculating the differences between the predicted and actual concentrations. The data achieved a measured dilution factor of 1.98 against the required dilution factor of 2 (Fig 2). Pipetting precision was high (i.e. %CV was low) across 5 repeats and was comparable between two different plate types for each dilution step (Table 1).

Plate type	Geiner Bio-One (784201)	SPT Labtech (LVSD)
	% CV per column	
Dilution 1	4	3
Dilution 2	3.6	2.6
Dilution 3	3	2.4
Dilution 4	5.7	2.9
Dilution 5	5	3.3
Dilution 6	6.2	2.9

Table 1. mosquito HV precision measured across 5 plates for two different plate types.

conclusion

The mosquito HV is accurate and precise for performing the serial dilution of very small volumes across different plate types.

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case study 2:

Cell cycle inhibition assay in 1536-well plate

method

5 µL of trypsinised HeLa cells (60 cells/µL) were transferred into each well of a BD Falcon™ 1536-well assay plate (BD Biosciences) using the mosquito HV and incubated overnight at 37°C.

In addition, one column of a 384-well plate was loaded with 10 µM stock solutions of four drugs (vinblastine, staurosporine, anisomycin and nocodazole). Diluent (culture medium) was added to the neighbouring two columns and a halflog dilution series was set up using the mosquito HV serial dilution wizard. 0.5 µL of these diluted drugs were then dispensed into the 1536-well assay plate preloaded with the HeLa cells and the plate was incubated for 24 hours at 37°C.

Cell cycle inhibition was measured by staining the cells with 0.5 µL of 10 µM Hoechst 34580 dispensed by the mosquito HV. The dye was incubated with the cells for 2 hours at 37°C and then the plate was scanned on the acumen® (SPT Labtech).

result

Nuclear staining of HeLa cells in the 1536-well plates was successfully illustrated using the TIFF views from the acumen's Cellista software (Fig 3). Higher concentrations of each of the drugs tested caused the cells to arrest in the G2/M phase of the cell cycle (Fig 3).

conclusion

These results present concentration-dependent inhibition of cell cycle progression by four specific drugs. This study also demonstrated the successful use of the mosquito HV for serial dilution in 1536-well plates.

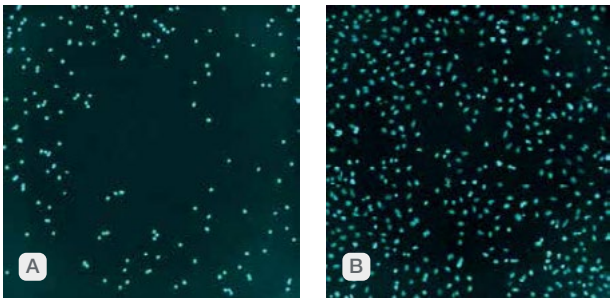

Figure 3. Nuclear staining of HeLa cells after treatment with nocodazole.

A. High percentage of cells in G1 phase of cell cycle after addition of lower concentrations of nocodazole. **B.** Reduction of cells in the G1 phase of the cell cycle after addition of higher concentrations of nocodazole.

case study 3:

Serial dilution for competitive binding FLISA bead assay

method

Unlabelled mouse IgG k1 (MOPC21) was added to the first column of a 384-well source plate and the diluent (Trizma, 10 mM, pH 7.0) was loaded into the second column. Using the mosquito HV serial dilution wizard a half-log dilution series of 10 points was dispensed in the neighbouring columns, mixing 5 times after each dilution. The mosquito then transferred 3 µL of each unlabelled antibody dilution to a 384-well flat bottom plate using the plate stamp step, followed by 3 µL of the competing Alexa Fluor 647-labelled mouse IgG.

Finally 42 µL of beads coated with anti-mouse IgG antibody (10 nM) were manually pipetted into the assay plate and competition between unlabelled and Alexa Fluor 647-labelled mouse IgG for the beads was quantified after 1 hour incubation at 4°C using a mirrorball® microplate cytometer (SPT Labtech).

result

A competition binding curve was generated for the unlabelled IgG antibodies (Fig 4). This can be utilised to determine the concentration of unlabelled mouse IgG in the samples.

conclusion

Accurate pipetting of an antibody dilution series can be achieved with the mosquito HV, which is essential to ensure good quality data is determined from the calibration curve.

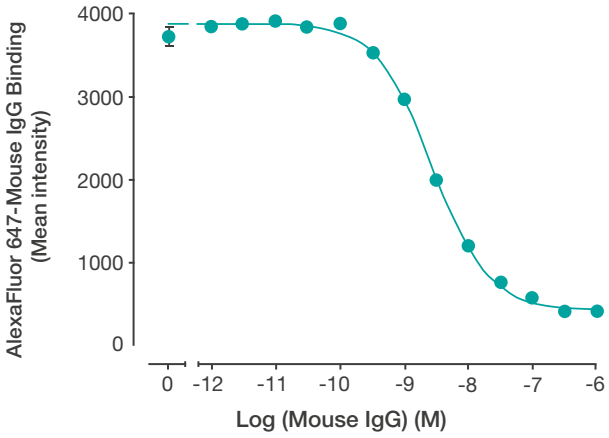

Figure 4. Concentration-dependent inhibition of Alexa Fluor 647 labelled mouse IgG (3.16 nM). Data are mean ± SD for 4 observations.

mosquito applicability for serial dilution

SPT Labtech's mosquito HV offers significant benefits for high throughput serial dilution:

- quick and easy to use, ideal for high throughput or multiple user labs
- different applications, plate types and liquid viscosities can be run in sequence without set-up changes or recalibration
- extremely accurate and precise pipetting through the 500 nL to 5 µL volume range
- improved data quality due to high pipetting accuracy and precision and zero cross-contamination
- reduced cost through miniaturisation of reagents used and near-zero dead volumes
- enviable reputation for robustness
- mosquito LV offers the same benefits to the lower volume range of 25-120 nL

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