

mosquito® HV: automated liquid handling of low volumes for biological assay and screen set-up

introduction

Operations such as aliquot transfer from stock plates, serial dilutions, plate preparation and replication all require accurate and repeatable pipetting, as well as meticulous sample labelling, tracking and management. Furthermore, the handling of low volumes employed in miniaturised assays significantly increases the potential for liquid handling error. In order to ensure high quality laboratory results, it is paramount to maintain accuracy and precision during the set-up of complex assays. It is unrealistic to expect this sort of time-consuming assay preparation to be performed manually in a high throughput format.

SPT Labtech's mosquito HV has a dispensing range of 500 nL to 5 µL which successfully bridges the gap between nanolitre and microlitre transfer requirements. This enables the automated set-up of standard biological assays without the need for special labware or instrument configuration changes. mosquito HV offers extremely fast and accurate plate replication and serial dilutions. This makes it particularly applicable to workstation-based workflows. It is also capable of reformatting between different plate types in the same protocol – even from standard 96-well formats direct to high density 1536-well plates.

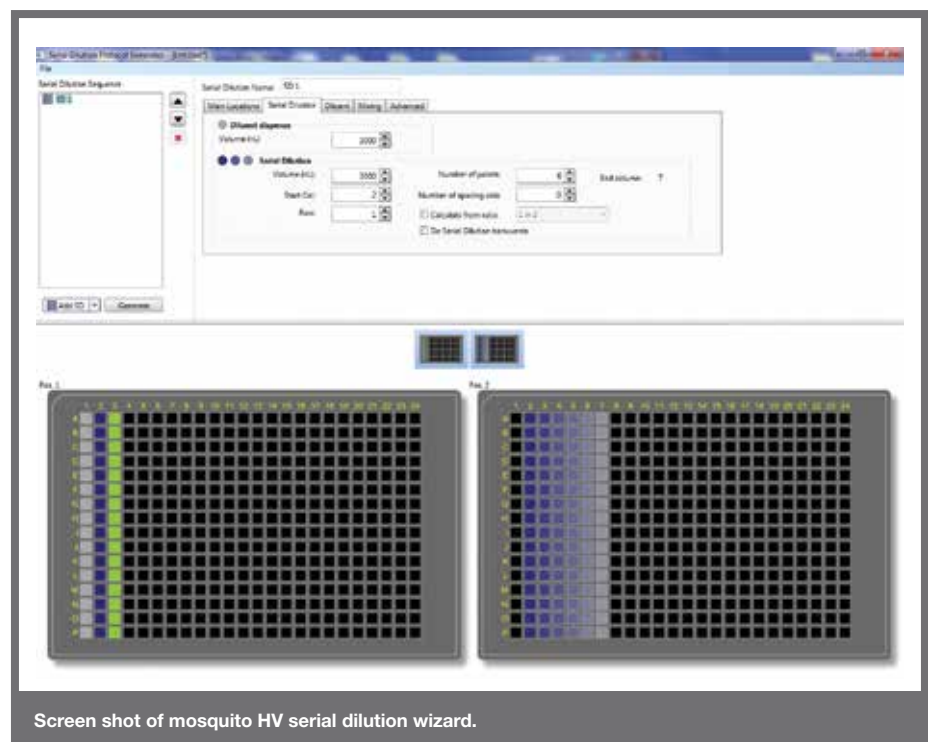
This technical note describes the protocol for the set-up of a bead-based assay using mosquito HV for the serial dilutions and assay plate creation.

cell treatment

- Tartrazine 2.18 mM (T0388, Sigma-Aldrich)
- Tris buffer 10 mM, pH 7.0, (T7193, Sigma-Aldrich)
- Avidin coupled beads 9.95 µm, (PC06N, Bangs Laboratories)
- Biotin labelled Goat anti-mouse IgG 200 µg/mL, (OSO2B-200UG, Oncogene)
- Mouse IgG1 k 1 mg/mL, (MOPC21, Sigma-Aldrich)
- FITC labelled mouse IgG1 k 1 mg/mL, (MOPC21, Sigma-Aldrich)
- Polyethylene glycol (PEG) 20,000 (Fluka, 81300)
- Matrical glass bottom 384-well microplate for mirrorball and Greiner 384-well standard plate (stock plate) and Greiner plate 784201 for serial dilution.

protocol and instrument set-up

As a preliminary study, mosquito HV was employed to perform a serial dilution of Tartrazine. mosquito HV pipetted from a 50 µL reservoir of Tris buffer (pH 7.0) and tartrazine stock (2.18 mM) in a 384-well plate. mosquito's serial dilution wizard was used to configure a two-fold dilution series involving the transfer of 3 µL of sample and buffer, starting from the stock concentration. The 'mix and move' function ensured appropriate mixing for each dilution. Following mixing, the tips were automatically discarded and replaced for each subsequent dilution, ensuring no cross-contamination.



Screen shot of mosquito HV serial dilution wizard.



SPT Labtech's mosquito HV

results

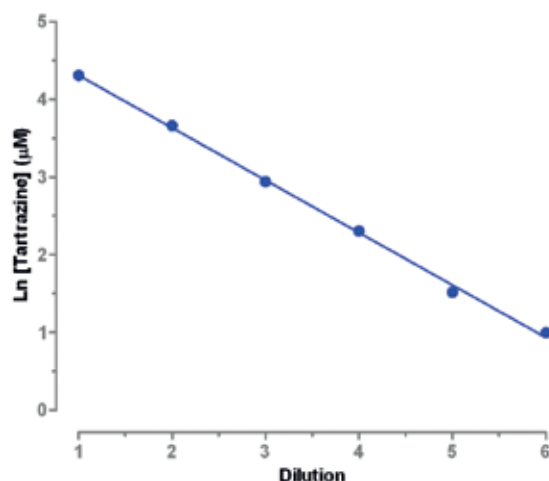


Fig 2. Linearity of dispensing a two-fold serial dilution of Tartrazine (2.18 mM) measured at 430 nm on a FLUOstar Optima (BMG Labtech).

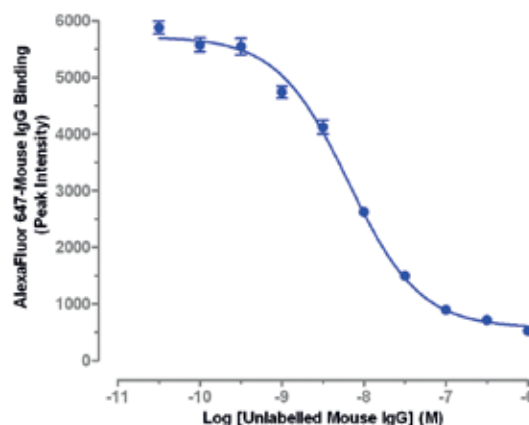


Fig 3. Concentration-dependent inhibition of Alexa Fluor 647 labelled mouse IgG (3.16 nM). Data are mean $\pm$ SD for quadruplicate observations.

set-up of a competitive binding FLISA for a mix-and-read bead-based format

This study demonstrates the use of mosquito HV to set-up a competitive binding FLISA to determine the concentration of mouse IgG in a mix-and-read format. Here, mosquito HV was employed to establish half-log serial dilutions mouse IgG. Competition with Alexa Fluor 647-labelled mouse IgG binding to goat anti-mouse coated beads was quantified after a 1 hour incubation using a mirrorball® microplate cytometer (SPT Labtech).

- 1 Avidin coated beads (50 μ L, approximately 2,000/ μ L) were mixed with biotin labelled goat anti-mouse IgG antibody in 450 μ L Tris buffer (10 mM, pH 7.0) and incubated for 90 minutes.
- 2 Beads were washed with Tris buffer containing 10% w/v PEG 20,000. Beads were re-suspended in 250 μ L Tris buffer containing 10% w/v PEG 20,000.
- 3 Unlabelled mouse IgG k1 (MOPC21) was serially half log diluted from a starting concentration of 395 nM in a V-bottomed Greiner plate.
- 4 3 μ L of each diluted antibody solution was transferred to the assay plate (plate stamp step). 3 μ L per well of labelled antibody was then added to the assay plate (12.5 nM) followed

by manual addition of buffer/beads combination (42 μ L). The plate was incubated at 4°C for 1 hour before scanning using mirrorball (SPT Labtech, 488 nm laser).

conclusion

Designed specifically for accurate sample dispensing within the volume range 500 nL to 5 μ L, SPT Labtech's mosquito HV offers you the following:

- versatility to perform high speed and accurate microlitre pipetting with a volume range suitable for scale up, technology and assay-ready plate preparation

- highly accurate and precise pipetting using positive displacement technology
- guaranteed zero carry-over between samples using disposable micropipettes
- easily integrated into laboratory automation, mosquito HV's volume range is compatible with pre-existing high content screening and HTS cell-based assays.

specifications

mosquito liquid handlers have been integrated with a wide variety of other laboratory instrumentation, including robotic plate handlers, stackers and bulk liquid handlers, to achieve walk-away operation in many application areas.

specifications	mosquito® HV
dispense range	500 nL - 5 μ L
plate capacity	up to 5
plate format	96-, 384-, 1536-well
dead volume	50 nL
min accessible volume	<1 μ L
dimensions (wxdxh)	390 mm x 470 mm x 690 mm (16" x 19" x 27")
weight	27 kg (59 lbs)
services	110/220 V single phase 50/60 Hz
noise	64 dBA peak noise during operation

*Capacity depends on manufacturers' specifications.

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for discovery



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