

Spotting of Meso Scale Screening Plates using a mosquito® Nanolitre Pipettor

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Abstract

Protein kinases represent the major target class for oncology research due to their prominent regulatory role in many cellular pathways, such as those leading to proliferation, differentiation and apoptosis. Historically, HTS of protein kinases has been performed through the combination purified enzyme and substrate. Such an approach investigates protein activity in a well controlled manner, but its overall relevance to protein function in the whole cell environment may be questioned. Thus there is an ongoing drive towards introducing cell-based HTS assays.

Meso Scale Discovery (MSD) have developed a platform capable of measuring the endogenous phosphorylation of a range of kinase targets in a variety of cell types. The technology consists of the combination of electrochemiluminescence detection and patterned arrays located in SBS format microplates.

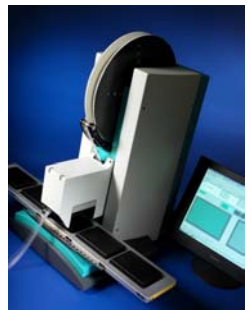
Electrochemiluminescence detection offers a unique combination of sensitivity, dynamic range and convenience. A prerequisite for developing these assay types is the selection of appropriate antibodies and optimisation of their concentration. For in house development this requires ability to accurately spot small volumes (~0.5µL) of capture antibodies onto the electrode in the centre of the well of the MSD plate.

mosquito® is TTP LabTech's compact nanolitre liquid handling solution. Here, we present data for its use for the manufacture of MSD plates

Conclusion

- mosquito's unique positive displacement disposable tips and precise X, Y and Z movements allow spotting of MSD 384 well plates with high accuracy and precision.
- The plate preparation time of 3 minutes per plate is compatible with screening demands
- The high pipetting and positional accuracy of mosquito makes it an ideal solution for manufacture of MSD plates in both small batches for assay development and larger quantities for screening.

1 mosquito® instrument

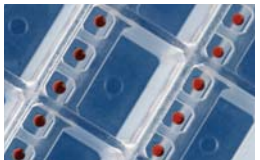


mosquito® is a low volume liquid handling instrument combining a low-cost disposable tip system with a positive displacement pipette to ensure zero cross-contamination.

mosquito is capable of pipetting volumes from 1.2 µL down to 50 nL with no washing required.

2 Accuracy of drop placement

mosquito's X, Y and Z axes are accurately driven by stepper motors with a resolution of <0.05 mm. This, along with the tightly toleranced and relatively short pipette tips, means that drops can be placed with a high degree of accuracy in the centre of wells of any SBS plate, up to and including the high density 1536 format.



Mosquito is routinely used for spotting protein for crystallography studies.

mosquito's accurate movement and disposable pipettes offer the following advantages:

- 50 nL to 1.2 µL aspirate and dispense range.
- Positive displacement pipetting handles liquids of varying viscosities accurately without recalibration.
- Disposable pipettes guarantee zero cross-contamination.
- Excellent repeatability and accuracy. Mosquito offers CVs of <8% at 50 nL and <4% at 100 nL across a 384 well plate; accuracy is within +/-5% throughout the volume range.
- Negligible dead volumes reduce sample wastage.

3 Electrochemiluminescence and MSD® Technology

MSD's products are based on MULTI-ARRAY® technology, a combination of electrochemiluminescence detection and patterned arrays (see www.meso-scale.com for further details). Virtually all experimental protocols use selective antibodies to detect analytes such as cytokines, growth factors or hormones or cell signalling events (e.g. protein kinase activity). This requires deposition of small volumes of each antibody in defined locations within the microplates during assay kit manufacture by MSD. An ability for users of the technology to construct their own spotted plates for bespoke assays yields several advantages based around time savings, flexibility, and sometimes cost.

- No requirement for shipment of proprietary antibodies to an external supplier saving time and cost
- Ability to optimise reagent evaluation and assay conditions to a final, ultimately multiplexable, format (i.e. without the use of a secondary capture reagent) more quickly, and according to internal procedures
- Spot on demand is more cost and time effective for small numbers of plates
- Simpler stock inventory of bare plates and capture reagents for spotting.

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4 Mosquito protocol for spotting 384-well small spot MULTI-ARRAY plates



3 minutes to spot one 384-well singleplex plate using 16-way tips

5 Spotting performance for 384-well small spot MULTI-ARRAY plates

Test Procedure:

- 384 well High Bind small spot MULTI-ARRAY plate
- 2 x spotting solutions – Integrin and antibody (unspecified)
- Coating volume 0.5 µL
- Pipettor – mosquito HTS (3 minutes per plate)

For integrin plates:

- Add 25 µL of 10 nM biotinylated fibronectin + 0.33 µg/mL ruthenylated-streptavidin solution diluted in MSD® integrin assay buffer
- Incubate
- Add 10 µL of read buffer T and analyse.

Protein	Whole Plate		%CV		
	Mean	SD	Plate	Column	Row
Integrin	76500	7897	10.3	9.0	8.7
Antibody*	65445	3944	6.1	4.7	4.8

* data are mean for two experiments.

6 Assay performance for coated 384-well small spot MULTI-ARRAY plates

Test Procedure:

- 384 well High Bind small spot MULTI-ARRAY plate
- Spotting solution – Integrin
- Coating volume 0.5 µL
- Pipettor – mosquito HTS (3 minutes per plate)
- Plate was assayed with both total and NSB wells (n=16)

	Total Signal	NSB
Mean	75373	674
SD	2707	36
%CV	3.6	5.3
Plate Z'	0.89	