

Robust nanolitre liquid handling for flexible assay miniaturisation

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introduction

Serial dilutions, plate replications and reformatting into higher density plates are important and often routine processes in drug discovery but, when assay volumes are miniaturised to nanolitre levels, there are some significant challenges to be met in the process of automating them.

Miniaturised assays have important cost benefits in the reduction of precious compounds and reagents required. As volumes are miniaturised, however, even small inaccuracies are proportionally magnified. It is therefore paramount to maintain accuracy and precision to ensure high quality laboratory results at these volumes or the efficiencies achieved become outweighed by the disadvantages. Miniaturising assays also allows users to draw on a range of high density plates for further efficiencies, but these can present challenges for liquid handling systems.

Other important criteria, though often overlooked, are the ease of use and reliability of the instrumentation. If a liquid handler is the key instrument in your process (which it often is) then you need it to have as little down time as possible, whether that is for re-programming, set-up changes, recalibrating or repair.

mosquito[®] HTS (TTP Labtech) is a nanolitre liquid handler flexible enough to automate the miniaturisation of all standard HTS assays without needing special labware or system set-up changes. It offers extremely fast and accurate plate replication and serial dilutions. 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.

1. mosquito instrument

mosquito HTS is a low volume liquid handling instrument combining a compact, low-cost disposable tip system with a positive displacement pipette. mosquito is capable of pipetting volumes from 1.2 µL down to 25 nL with no washing required and extremely rapid tip changing.



2. accuracy and precision

mosquito is unique in using positive displacement disposable pipettes, fed into a fixed head over a movable plate deck. This combination offers the 3 key requirements for assay miniaturisation: accuracy, precision and repeatability.

mosquito's pipetting achieves average CVs of less than 2% across the volume range; with accuracy with an average of 3%.

The positive displacement micropipettes are consistently accurate across a wide range of fluid viscosities without the need for frequent recalibration. The table below shows CVs at the lower end of the pipetting range for a very wide range of viscosities.

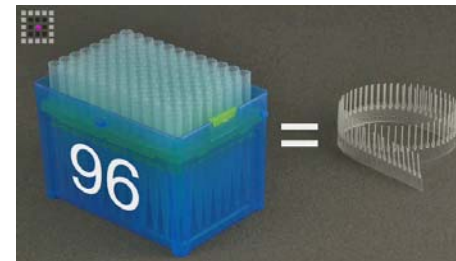
Solution	Vol	CV
0.2M ammonium acetate, 0.1M sodium citrate pH 5.5, 30% (w/v) PEG 4,000	100nL	2.8%
0.2M magnesium chloride, 0.1M HEPES pH 7.5, 30% (v/v) isopropanol	100nL	5.5%
PBS	100nL	2.7%
0.1M HEPES pH 7.5, 70% (v/v) MPD	100nL	2.8%
40% PEG 20,000, 96mM TRIS pH 7.1	100nL	5.5%
100% DMSO	100nL	3.9%

3. improved efficiency

Miniaturising assay volumes and reducing dead volumes optimises reagent use – customers quote a 16-fold reduction in compound use.

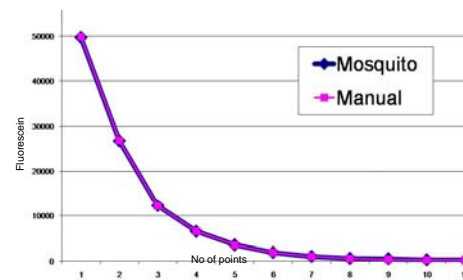
mosquito's disposable pipettes are also miniaturised which improves efficiency in the following ways:

- ✓ no in tip dead volumes
- ✓ minimal dead volumes in source wells (<500 nL)
- ✓ no wash steps and no cross-contamination
- ✓ very space-efficient for storage/disposal



4. two approaches to generating low volume concentration-effect curves

mosquito HTS is capable of performing either direct volumetric dose response assays, or of miniaturising traditional serial dilutions without set-up changes.



Comparison of mosquito versus manual pipetting for a 1 in 2 11-point serial dilution with 200 nL stamp out into tissue culture treated plate (difficult due to DMSO wicking)

5. flexibility

mosquito HTS is simple to use for different applications:

- No set-up changes or recalibration for different protocols, plates or liquid types are required. Users simply swap between reformatting, plate replication, serial dilutions or assay-ready plate preparation by changing the protocol.
- Use different plate formats in the same protocol and reformat between different plate types. mosquito addresses SBS-format plates from any manufacturer.

96 well		384 well		1536 well	
96 to 96	yes	384 to 96	yes	1536 to 96	yes
96 to 384	yes	384 to 384	yes	1536 to 384	yes
96 to 1536	yes	384 to 1536	yes	1536 to 1536	yes

6. throughput

mosquito HTS incorporates extremely rapid tip changing with a deck holding up to 5 plates. It offers high-throughput processing across the entire volume range. It can generate 384-well daughter plates in 1.5 minutes/plate, or perform a full 96-well plate 11-point serial dilution in only 1.5 minutes.

conclusion

- Sample volumes as low as 25 nL can be applied at high precision resulting in conservation of stock compound solutions.
- Miniature disposable pipettes also minimise waste such as dead volumes in well and tip, and eliminate wash solvents and steps.
- Users can quickly swap between applications, plate types and liquid viscosities without set-up changes or recalibration.
- Users can choose between two accurate methods of generating miniaturised dose response curves.
- mosquito has proven to be reliable and robust, even in multi-user environments, with minimal down time.

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