

Improving the efficiency of 384 “mini-tube” technology using mosquito® nanolitre pipetting

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Introduction

384-well plate “mini-tube” consumables have been developed by automation companies such as REMP and The Automation Partnership (TAP) to hold compound samples divided into single-use storage tubes. Each tube holds 40 -75 μL , and is filled, sealed, stored and then thawed just once before use. However, when accessed by conventional pipetting technology, the tube's practical working volume is as little as 20 μL , which means high compound wastage. The seal must also be pierced prior to pipetting to provide access to the sample.

TTP LabTech's mosquito® liquid handler uses positive displacement pipetting which makes it uniquely suitable for this niche application. The disposable micropipettes can pierce foil or plastic seals, so there is no need for standalone piercing equipment. The micropipettes can reach to the bottom of 384 mini-tubes, leaving minimal dead volume. Mosquito is also able to dispense nanolitre quantities in order to create multiple miniaturised confirmation assays or serial dilutions from hit samples.



Conclusion

Key benefits of mosquito:

- nanolitre volume working range.
- compatible with working volumes of 2 μL – virtually eliminating compound wastage.
- piercing action is a function of sample retrieval, avoiding the need for separate piercing equipment.

The mosquito® can provide HTS with the capability of making multiple serial dilutions directly in assay plates with only the 2 μL sample that is provided in the mini-tube for HTS confirmations. The 10 μL tubes for IC50 value determinations can then be used for further assays. The ability to perform IC50 values on confirmation samples eliminates a step in the screening process, as does removing the need to pre-pierce tubes.

1 384 mini-tube Technology

The 384 “mini-tube” consumables have been developed to reduce sample degradation which can occur with multiple freeze-thaw cycles when accessing samples within conventional storage containers.

Using mini-tubes allows compound samples to be divided into multiple, individual storage tubes, each containing just the right amount for single use and thawed once just before use, instead of being stored in a traditional, larger volume container.

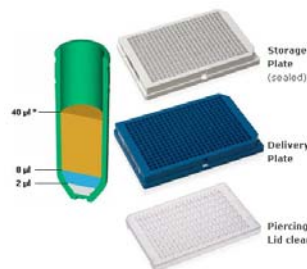
Samples in mini-tubes can be arranged in a 384-well plate format ready for onward processing.

The recommended working volume for most mini-tubes is around 40-75 μL with a dead volume of 2 μL .

This tube format has advantages for sample storage but can create problems for automated liquid handling systems.

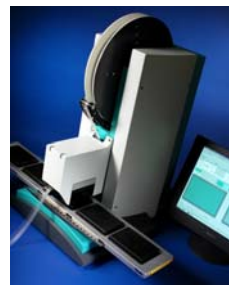
Disadvantages include:

- high compound wastage. The practicable working volume appears to be about 20 μL as instruments using conventional pipettes find it difficult to reach the bottom of these tiny tubes
- storage of volumes < 20 μL requires addition of diluent for retrieval and hence reduction of stock concentration
- piercing is necessary for tube access. For example, REMP, a major supplier of 384 Tube Technology, offer Manual (shown in the diagram above), Universal or Automated Plate Piercers which operate as standalone devices.



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2 mosquito® Instrument



TTP LabTech's mosquito® uses low-cost disposable micro-pipettes to ensure zero cross-contamination.

These pipettes are capable of aspirating, dispensing and mixing volumes from 1.2 μL down to 50 nL. Using positive displacement ensures accurate and reproducible pipetting.

mosquito's pipettes consist of a stainless steel piston in a close-fitting HDPE barrel. These inert materials have no effect on most reagents and are strong enough to pierce common plate sealing films as part of sample retrieval, so there is no need for separate piercing equipment.

Mosquito's pipettes are only 0.8 mm diameter so can reach to the bottom of 384 tubes leaving less than 800 nL in dead volumes.

Since the pipettes can directly access the bottom of mini-tubes (with minimal displacement of the contained liquid), no additional diluent is required and the top stock concentration is maintained in the mini-tube.

Hit compounds in mini-tubes can be cherry-picked and arranged in the final 384 microplate layout. These plates can then be transferred directly to the mosquito® deck so mosquito® can pipette volumes down to 50 nL to a dry microplate for secondary confirmation or carry out low volume serial dilutions.



3 Validation Workflow – Secondary Screening

1. Compound library stored as 2 μL samples using 384 mini-tube consumables.
2. Hit compounds from the primary screen were cherry-picked and arranged in 384 microplate format.
3. 384 plates were transferred to mosquito® plate deck for compound retrieval.
4. mosquito® micropipettes pierced the foil-sealed tubes and transferred 50 nL to a dry 384 microplate for secondary confirmation.
5. Subsequently, mosquito® transferred a second sample to a 384 microplate for serial dilution for dose dependency determination.

4 Validation Data

1) Hit Confirmation: 50 nL Transfer

Note: All compounds were dissolved in DMSO

Compound	Measurement	Plate type	% CV
2% Tartrazine	Absorbance	Greiner	7.3
		Matrix	9.4
50 μM Fluorescein	Fluorescence	Greiner	5.0
		Matrix	4.8

2) Serial Dilution: 158 nL Transfer

Total dilution volume was 500 nL. For half-log dilution of fluorescein volumes were 158 nL Stock + 342 nL diluent. Data are mean \pm SD (n=16).

