



mosquito[®] crystal, mosquito[®] LCP & an active humidity chamber: essential tools for successful membrane protein crystallisation

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introduction

The automation of protein crystallography screening has contributed significantly to the rapid progress of crystallography-based structural biology offering increased throughput and accuracy, with the ability to use smaller volumes of both protein and screen solutions, thereby saving valuable protein and reducing reagent costs.

Automation of protein crystallisation trials set-up requires accurate placement of nanolitre volumes of protein and screen drops, in addition to the reproducible and accurate dispensing of solutions of varying viscosities. This is particularly important for the set-up of the highly viscous lipid mesophases in the LCP crystallisation technique for membrane protein crystallisation trials.

mosquito[®] LCP by TTP Labtech offers a fully automated solution to LCP screen set-up. With an optional LCP mixer and a microsyringe dispenser that can accurately dispense nanolitre volumes of the highly viscous cubic phase, mosquito LCP offers fast throughput, high precision and unrivalled reproducibility. mosquito's positive displacement technology and disposable tips ensure unrivalled accuracy and zero-contamination between samples.

This poster describes the features of TTP Labtech's mosquito[®] crystal and mosquito LCP and their ability to address the issues of the automated set-up of protein crystallisation screen trials. The ability to automate both micro batch and vapour diffusion methods of protein crystallography (sitting drop, hanging drop) without instrument configuration change offers significant flexibility for the crystallography laboratory. mosquito LCP offers all the advantages of mosquito crystal but with the addition of a dedicated microsyringe dispenser for accurate dispensing of nanolitre volumes of the highly viscous cubic phase.

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1. mosquito LCP instrument

mosquito LCP is a low volume liquid handling instrument that has been specifically designed to overcome the challenges presented by the LCP method and provide an automated solution to LCP screening techniques.

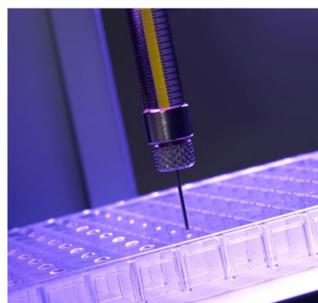
LCP is highly viscous, thus difficult to manipulate. This in turn makes the automation and the miniaturisation of the LCP crystallisation set-up very challenging.

With its unique features, mosquito LCP solves these problems.



2. mosquito LCP features

- accurate and repeatable dispensing of LCP down to 25 nL using a dedicated positive displacement syringe.
- automatic measurement and calibration of the LCP syringe needle tip position to within +/- 20 µm enables precise drop placement and subsequent automated imaging of membrane protein drops and crystals



- choice of either 50 µL or 100 µL capacity syringe
- loading/changing of syringes is facilitated via user friendly, wizard-based software.
- software wizards enable simple definition of plate types, number of columns, and volumes of LCP mixture and screen solutions to be pipetted.
- disposable, positive displacement mosquito tips guarantee zero cross contamination and eliminate time-consuming tip washing.
- minimized evaporation of the dispensed LCP as a result of screen solutions being dispensed on the columns of LCP within 5 seconds.
- plate set-up time of less than 5 minutes results in high throughputs of >12 plates an hour.

3. calibration

mosquito LCP is able to accurately detect and calculate the position of the central axis and tip of the LCP dispensing needle in the X, Y and Z axes. Calibration is achieved using a crossed pair of photoelectric sensors mounted directly on the mosquito deck.

Proprietary electronics drive and control the Z height positioning of the LCP syringe needle tip with precision accuracy with repeatable positioning with 5 µm being achievable.



Positioning of the LCP dispense needle relative to the mosquito deck is accurate to +/- 20 µm in the X axis and +/- 5 µm in the Y and Z axes.

The electronics also enable rapid and highly accurate loading of a new syringe. Users can directly drive the syringe pump into the correct loading position without having to refer to the user interface on the screen as a result of the addition of the button pad interface for syringe adjustment.

4. LCP dispensing precision & accuracy

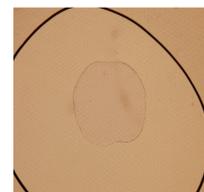
Repeatability:

- at 100 nL, CVs of < 6%
- at 50 nL, CVs of < 8%
- at 25 nL, CVs of < 10%

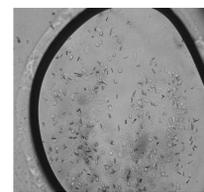
Accuracy:

- Within 10% accuracy at volumes of 25 nL and higher

Fig 1.



LCP 'sandwich' drop image



Crystal image supplied by Tony Warne, MRC, London

5. typical protocol for LCP screening set-up

- a 96-well screen plate and LCP 'sandwich plate' are loaded on to the deck of mosquito LCP.
- a syringe containing the pre-mixed LCP/protein solution is loaded on to mosquito LCP using the 'Change Syringe Wizard'.
- tip position is automatically calibrated to high accuracy to reference its exact position in X, Y and Z dimensions.
- the required protocol is selected or a new protocol is generated using wizard software.
- step 1: a column (8 wells) of LCP mixture is dispensed, one well at a time. This process takes <20 secs.
- step 2: a corresponding column of screen solution is aspirated from the source plate and dispensed on top of the LCP drop using the positive displacement tips in the mosquito head. This process takes ~5 secs.
- steps 1 and 2 are repeated for all 12 columns of the plate.
- the cover sheet of the LCP sandwich plate is applied using an alignment jig, to seal the LCP/screen drops in the wells. Figure 1 shows the LCP screen drops and resulting crystals.
- the overall set-up time for a 96-well plate is <5 mins.

conclusions

- sample volumes as low as 25 nL can be applied at high precision resulting in conservation of stock compound solutions.
- mosquito LCP offers a fully automated solution to LCP screening set-ups.
- it ensures accurate and repeatable dispensing of LCP down to 25 nL.
- the accurate syringe and pipette positioning permits precise drop-on-drop placement of the LCP solution which facilitates automated imaging
- mosquito LCP has all the functionality of the standard mosquito crystal and enables zero loss dispensing of protein solutions or other expensive/highly viscous additives
- this instrument enables both LCP and traditional crystallisation experiments to be set up in commercially available plates.
- mosquito LCP enables high throughput screening set-ups for membrane proteins such as G-protein-coupled receptors

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