



Wizard CUBIC™ LCP Kit

U.S. Patent N. 6,821,487

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The Wizard Cubic™ LCP kit is based on the pioneering work of J. Rosenbusch and E. Landau who demonstrated that soluble and membrane proteins can be crystallized within a Lipidic Cubic Phase (LCP) matrix (1). This Wizard: Cubic™ LCP Kit contains everything needed to set up micro crystallization experiments following the LCP method (2, 3, 4, 5, 6). Conceptually, the technique consists of two steps: first, biological macromolecules are incorporated into a lipidic material, the Lipidic Cubic Phase, and in a second step the LCP is dispensed in portions of approximately 200 nanoliters. The LCP forms spontaneously and acts as a matrix supporting the crystallization of soluble and membrane proteins. Due to the high viscosity of the LCP, the material is manipulated using positive displacement syringes. The detailed protocol below explains the preparation of the LCP, its dispensation, and the preparation of crystallization experiments.

Your LCP Kit Contains:

- Two 250 μ L syringes with a Mixer Union for the preparation of LCP
- One ratchet dispenser with 10 μ L syringe and short needle to dispense the LCP
- One 10 μ L Microsyringe Pipette
- 100 mg Monoolein
- Ten micro trays
- Cubic™ Screen formulation matrix block
- Spatula and tape

Protocol Step 1

Preparation of the LCP within a Syringe: Mixing Protein Solution with Lipid to Obtain LCP

Attach the Mixer Union to one of the two 250 μ L Syringe (Syringe 1). Make sure that the large white Teflon ferrule sits on the needle end of the Mixer union with the large steel barrel before it is inserted and thread onto Syringe 1. Remove plunger from Syringe 1.

1.1 Weigh Lipid (Monoolein) into 250 μ L Syringe

Place this Syringe 1 assembly on a balance (recommended precision 0.1 mg), and tare. Using the small spatula, transfer approximately 60 mg of powdered Monoolein into the open glass barrel of Syringe 1. Note precise mass, insert plunger and compress the lipid to remove air gaps within the lipid powder.

Notes:

- Powdered Monoolein can be generated from a solid by breaking open the ampoule and scraping off the solid lipid, creating small powdery flakes.
- To keep the lipid from melting it is advisable to maintain a temperature below 34°C. Store the Monoolein at -20 °C in a gas tight container; wait for equilibration to room temperature before opening the vial; keep from moisture.

1.2 Add Protein Solution to Syringe 2 and Join with Syringe 1

Fill approximately 40 μL s of protein solution into 250 μL Syringe 2, i.e. by pipetting the liquid through the orifice of the syringe. Push the plunger up into Syringe 2 and make sure to exclude or to remove air bubbles. Add the second Teflon ferrule to the Mixer Union and connect Syringe 2 to the Syringe 1 assembly. Expel any additional air before final tightening of the assembly.

1.3 LCP Formation by Mixing

Carefully plunge contents of Syringe 2 (protein solution) into Syringe 1 (lipid) wetting the lipid. It may take several strokes before the entire content of one syringe can be passed into the other syringe. Continue pushing the mix back and forth through the Mixer Union until a homogenous blend is obtained (i.e. 100 passes). Transfer all of the content into Syringe 1.

Notes:

- Do not bend the assembly
- The resulting LCP material should be transparent, highly viscous and non-birefringent (can be tested using a microscope and crossed linear polarizers).
- When prepared at temperatures above 20 $^{\circ}\text{C}$ or at higher water content, the initial mix is turbid and turns clear after short cooling and repeated mixing.

Protocol Step 2

Setting up the Crystallization Experiment: Dispensation of the LCP

2.1 Loading: Transfer of LCP from 250 μL Syringe into 10 μL Syringe

Mount the 10 μL Syringe onto the ratchet dispenser (tighten ring gently) and push plunger into the syringe, leaving it unattached. Remove Syringe 2 from the mixer assembly, exposing the small Teflon ferrule and the small steel barrel. Use the Mixer Union to couple Syringe 1 to the 10 μL syringe. Carefully transfer a portion of LCP (i.e. 10 μL) from the 250 μL Syringe 1 into the 10 μL syringe by pushing the plunger of the 250 μL Syringe. Simultaneous manual guiding the retracting plunger of the 10 μL syringe assures control of this delicate filling procedure.

2.2 Complete Assembly of the Ratchet Dispenser

Remove the 250 μL Syringe from the assembly and attach the small dispensing needle to the 10 μL microsyringe with the nut (transfer the small Teflon ferrule from the Mixer Union to the short portion of the small needle prior to insertion). Pressing the dispenser button will extrude LCP through the needle. The Ratchet Dispenser assembly is primed by pressing the dispenser button several times until portions of LCP material appear at the needle tip. Clean needle tip with tissue.

Note:

- The 10 μL micro syringe requires filling every approximately 45 dispensations

2.3 Prepare Crystallization Experiments: Dispense Crystallant and LCP

Transfer 2 μL aliquots of CubicTM Screen formulation into wells of the micro tray using the 10 μL microsyringe (this may be done in rows of 6; to prevent cross contamination quickly wash micro syringe pipette between dispensations with water and wipe dry). Inject 0.2 μL of LCP into each pre-filled drop chamber using the Ratchet Dispenser.

Note:

The goal of this step is to produce and attach a 'slug' of LCP on the bottom of the micro well. This can be done like this: Hold the Ratchet dispenser with your right hand, placing the thumb onto the button; hold the tray with your left hand. Insert the dispenser at a steep angle but not vertically; allow the needle to firmly contact the tray bottom at the edge of the well. Press button while needle remains in contact with the bottom and, after 'slug' has been extruded, retract the assembly along the direction of the needle. This procedure assures proper 'slug' formation and prevents the LCP from sticking to the needle. Make sure not to 'scoop up' the formed LCP 'slug' with the needle.

2.4 Seal Crystallization Experiments

Seal crystallization chambers with tape. This may be done in batches of 6 wells (i.e. individual rows).

2.5 Inspection and Crystal Manipulation

The crystallization experiments may be inspected from both sides using a conventional stereomicroscope. When tape is used as a sealant the plates may be flipped upside down in order to enhance the visibility of the LCP. Crystals may form in the bulk of the LCP, in the liquid portion or in both. Crystals may be removed directly or after liquefying the LCP.

References

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