For growing large protein crystals by dialysis and diffusion

### Practical – Growing large Lysozyme crystal

1. Keep the gel-tube of C-Tube LCDM in 5% PEG 4000, 0.4 M NaCl and 50 mM acetate buffer pH 4.5 to soak the solution.

2. Load 25 mg/ml lysozyme, 5% PEG 4000 and 50 mM acetate buffer pH 4.5 into the C-Tube LCDM cell with the fine top tip.

3. Reservoir solution #1: 5% PEG 4000, 0.4 M NaCl, 50 mM acetate buffer pH 4.5. A small crystal starts growing (Fig. 1).

4. Change the reservoir solution from #1 to #2: 5% PEG 4000, 0.7 M NaCl, 50 mM acetate buffer pH 4.5. The crystal is growing larger (Fig. 2).

5. Change the reservoir solution from #2 to #3: 25% PEG 4000, 0.7 M NaCl, 50 mM acetate buffer pH 4.5. The crystal is growing larger. By using higher PEG solution, X-ray diffraction data of higher resolution is expected (Fig. 3).

### C-Tube LCDM

#### Catalog # | Product name | Description | Price (w/o tax)
--- | --- | --- | ---
MB2004-CRT810 | C-Tube LCDM(RC) | Regenerated cellulose membrane (MWCO 6-8kD) 6 sets | Please contact us
MB2004-CRT820 | C-Tube LCDM(CE) | Cellulose ester membrane (MWCO 1kD) 6 sets | Please contact us
MB2004-CRT830 | C-Tube LCDM(PES) | Polyethersulfone membrane (MWCO 10kD) 6 sets | Please contact us

- **Crystallization cell**
  - Size: 2.5mm O.D., 1.92mm I.D., 18mm length
  - Material: Glass
  - Sample volume: 30μL
  - Dialysis membrane: Regenerated cellulose membrane (RC) MWCO 6-8kD; cellulose ester membrane (CE) MWCO 1kD; polyethersulfone membrane (PES) MWCO 10kD.
  - Pre-attached gel-tube.
- **Other items**: Gel soaking bag; crystallization bag; fine top tip; PTFE tubing tip; C-Cap.

#### Summary of X-ray diffraction experiment on lysozyme crystal

<table>
<thead>
<tr>
<th>Diffraction source</th>
<th>Aichi SR BL251</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wave length (Å)</td>
<td>1.12</td>
</tr>
<tr>
<td>Camera system</td>
<td>ADSC315r</td>
</tr>
<tr>
<td>Space group</td>
<td>P43 21 2</td>
</tr>
<tr>
<td>Unit-cell parameters (Å, °)</td>
<td>79.1, 79.1, 38.2, 90.0, 90.0</td>
</tr>
<tr>
<td>Average mosaicity (°)</td>
<td>0.07</td>
</tr>
<tr>
<td>Resolution range (Å)</td>
<td>39.54 – 1.39</td>
</tr>
<tr>
<td>(1.42 – 1.39)</td>
<td></td>
</tr>
<tr>
<td>Completenss (%)</td>
<td>99.9 (100)</td>
</tr>
<tr>
<td>Rmerge</td>
<td>0.028 (0.569)</td>
</tr>
<tr>
<td>&lt;I/σ(I)&gt;</td>
<td>41.4 (4.0)</td>
</tr>
<tr>
<td>Rmerge (I) half-set correlation (CC/1/2)</td>
<td>1.00 (0.923)</td>
</tr>
</tbody>
</table>

### The 5 Major Features of C-Tube LCDM

1. **Dialysis/diffusion method**
   - No protein loss during the crystallization experiment.
   - Osmotic pressure difference is small in C-Tube LCDM.

2. **Amount of protein sample**
   - 30μL of protein sample is required.
   - If V_m is 2.2Å^3/D, one crystal of 1.2mm^3 size can grow.

3. **Crystallization condition**
   - The crystallization condition which suppresses nucleation probability should be chosen, considering the diffusion of protein and precipitant molecules in the solution.
   - Please contact us for consultation and technical assistance.

4. **Easy preparation**
   - A gel-tube (a gel in silicone tubing) is pre-attached to the C-Tube LCDM. Preparation is easy.

5. **Long-term stability/space-saving**
   - The crystals grown in the cell are stable in long term.
   - Using the gel soaking bag and the crystallization bag, you can perform crystallization experiments in a small space.
How to assemble C-Tube LCDM

*Gel soaking*
C-Tube LCDM should be immersed into the proper gel-soaking solution in several days before loading protein solution. The gel-soaking bag is sealed by a heat sealer (option).

*Solution preparation*
Fill the crystallization bag with 0.5 mL of reservoir solution using a PTFE tubing tip.

*Picking up C-Tube LCDM*
After the gel tube is well-soaked with the gel-soaking solution, pick up C-Tube LCDM from the gel-soaking solution.

*Protein solution loading*
Load the protein solution into C-Tube LCDM with the fine top tip, and plug the upper edge of C-Tube LCDM into the C-Cap.

*Crystallization cell set-up completed*
Insert the C-Tube LCDM into the crystallization bag.

*Sealing the bag*
The crystallization bag is sealed by the heat sealer.

*Start crystallization*
Observe crystal growth.

*Instructional video*

Related item ‘C-Chip-DM’ : Gel-tube with dialysis membrane

- **MB2004-CRT901 C-Chip-DM15** / 6 pieces

**Features**
1. Crystallization reagent gradually diffuses into a capillary through the gel-tube. Mild crystallization condition can be realized.
2. Small amount of sample is required in this dialysis method.
   - 0.3mm I.D. and 30mm length in a capillary: 2.2μl
   - 0.5mm I.D. and 40mm length in a capillary: 7.9μl

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Please read the instruction manual carefully before using this product.

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