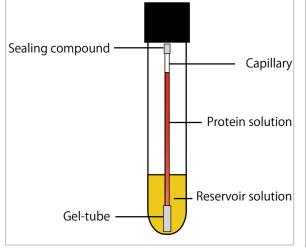
Crystal Tube Kit Counter-Diffusion Protein Crystallization Kit



Crystal Tube Features

- Size: Very little space is required for crystallization set-up.
- Easy Set-up: The Gel-tube already contains agarose in the silicon tube, making the crystallization set up very easy.
- Small Protein Sample: Only 2 micro-L per screening. Only 10 micro-L per diffractive-grade crystal.
- Favorable Crystallization Conditions: The timing of crystallization can be controlled by Gel-tube length, the amount of protein solution in a capillary, and the concentration of protein and precipitant solution.
- High Reproducibility and Reliability: JAXA uses this method for crystallization in space. After crystallizing over 400 different proteins, the Crystal Tube kit has proven its reliability and high reproducibility.
- Long-term Stability of Crystals: Crystals grown in the capillary are stable over the long term, making it easy to soak crystals with another compound, such as a ligand or cryoprotectant.
- Membrane Protein Crystallization: Phase separation due to concentrated detergent in the solution does not occur.
- User Manual: Download our user manual at http://www.confsci.co.jp/c-tube.html to learn about crystallization using counter-diffusion method.



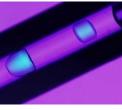
How Does It Work?

Counter-diffusion is a crystallization method in a capillary in which a protein and a reservoir solution diffuse into each other from opposite directions.^{1), 2)} Both the protein and the precipitant solutions are set to diffuse through the gel, and the concentration gradients of both solutions form in the capillary. The capillary can continuously scan a wide range of crystallization conditions unless crystallization occurs. By fixing the precipitant concentration higher, you can scan a wider range of crystallization conditions. Therefore, a single capillary may be equivalent to many drops in the vapor-diffusion method.

1) McPherson A., Crystallization of Biological Macromolecules, Cold Spring Harbor Lab. Press (1999) 2) Garcia-Ruiz, J.M., Moreno, A.: *Acta Cryst.*, **D50**, 484-490(1994)

Protein Crystals Grown in the Crystal Tube









Serine protease

Cellulase

HEW Lysozyme



Preparing Crystallization Conditions

1.	Choose a plausible buffer with a pH 1~2 departed from the pI of the target protein.
2.	Prepare the following reservoir solutions: • Buffer + 4M (NH ₄) ₂ SO ₄ • Buffer + 30 % PEG 4000 + 100 mM ~ 1 M NaCl
3.	Cut the Gel-tube to 10 mm and soak it in these solutions for a few days prior to set-up.
4.	Set up the Crystal Tube with the protein and the reservoir solution. 2 μ l protein solution for 0.3 mm capillary with 40 mm length; about 20 μ l is enough for the first trial.

Example

Buffer + 30% PEG 4000 + 0~700 mM NaCl

Protein		Lysozyme		alpha-Amylase			Glucose Isomerase	
рН		4.5	7	5.5	7	9	7	9
	0	clear	clear	clear	clear	clear	clear	clear
	100	clear	clear	С	clear	clear	clear	clear
	200	clear	clear	С	C,O	0	C,P	С
NaCl	300	clear	С	С	C,O	0	C,P	C,P
(mM)	400	С	С	C,O	0	0	C,P	C,P
	500	С	С	-	0	0	C,P	C,P
	600	С	С	-	0	0	C,P	C,P
	700	С	С	-	0	0	C,P	C,P

Conditions in which crystals, oil and/or precipitate were observed are indicated by "C", "O" or "P", respectively.

- There is an optimum salt concentration for PEG as the precipitant, as shown in the table. If too low, crystals will not grow. If too high, precipitate or oil will be obtained.
- Crystallization can sometimes be accelerated if the protein and the reservoir solutions are pre-mixed 1:1 before crystallization set-up.

Reference ICCBM12 (2008) Program & Abstracts Book 128

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Starter kit MB2004-CRT209 ¥15,000

If you are new to the Crystal-Tube, the starter kit will save prep time and ease crystal harvesting.

1. Software for optimization

- Estimation of the optimum salt concentration
- Simulation of the diffusive process in the capillary

2. Capillary Holder

• To hold the capillary when taking out a crystal.



Sales agency

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