

Crystal Dehydration and Salvage Kit

Cat. No.	Amount
CO-122	1 Kit

For *in vitro* use only
 Quality guaranteed for 12 months
 Store salt solutions at 4°C *

Application

The Crystal Dehydration and Salvage Kit is designed to modify and/or improve the diffraction properties of protein crystals.

Kit contents

The Crystal Dehydration and Salvage Kit comprises:

- 12 saturated salt solutions (Table 1), 1 ml each
- 6 GB-B3S goniometer bases (also referred to as "caps")
- 20 MicroRT capillaries

No.	Salt	Molecular formula	r.h.	Δ
1	Potassium sulfate	K ₂ SO ₄	97.3%	-60.0%
2	Potassium nitrate	KNO ₃	93.6%	-2.3%
3	Sodium benzoate	NaC ₇ H ₅ O ₂	88%	> 1%
4	Potassium chloride	KCl	84.3%	-1.5%
5	Ammonium sulfate	(NH ₄) ₂ SO ₄	81.0%	-0.7%
6	Ammonium chloride	NH ₄ Cl	78.6%	-1.3%
7	Sodium chloride	NaCl	75.3%	-0.4%
8	Potassium iodide	KI	68.9%	-2%
9	Sodium bromide	NaBr	57.6%	-3%
10	Potassium carbonate	K ₂ CO ₃	43.1%	> 0.1%
11	Magnesium chloride	MgCl ₂	32.8%	-0.6%
12	Potassium acetate	KC ₂ H ₃ O ₂	22.5%	-1.5%

Table 1: Relative humidities (r.h.) at 25°C and absolute variation Δ between 20°C and 30°C [1,2] for the saturated salt solutions in this kit.

Why Dehydrate Your Crystals?

Dehydration has been used as a tool for inducing structural changes in protein crystals since the earliest days of protein crystallography. Max Perutz [3-5],

John Kendrew and Hugh Huxley [6] used it in their studies of hemoglobin and myoglobin in the 1940's and 1950's. Francis Crick was supposed to work on dehydration when he joined Perutz's group, but suffered a famous distraction.

Dehydration remains a powerful — and underutilized — tool for improving or at least modifying the diffraction properties of protein crystals [7,8].

- Dehydration removes excess solvent, tightens packing of protein molecules, and reduces the size of solvent channels. As a result, it sometimes improves crystal order and diffraction resolution.
- By removing excess solvent, dehydration can make successful flash cooling easier, especially for crystals with large initial solvent contents.
- When sufficiently dehydrated, many protein crystals undergo structural transformations, yielding alternative crystal packings that may be difficult or impossible to achieve directly during crystal growth.

Of all post-crystallization treatments, dehydration has proven to be the most effective in improving crystal diffraction properties [9]. Of course, dehydration also often severely degrades crystal diffraction, but (amazingly!) original crystal order can usually be fully recovered just by rehydrating.

The Crystal Dehydration and Salvage Kit

Saturated salt solutions are the basis for an easy, controlled and reliable way to dehydrate protein crystals.

When placed in an enclosed container, a saturated salt solution will maintain a fixed relative humidity above it, even if water evaporates from the solution and container. As shown in Table 1, saturated solutions of different salts produce different relative humidities.

* A salt precipitate might be observed at 4° C. This is no shortcoming of the product. Simply, warm up the vial until the precipitate dissolves.

Crystal Dehydration and Salvage Kit

By equilibrating a crystal via vapor diffusion with a series of saturated salt solutions, the crystal's solvent content can be reduced in a controlled and reproducible way. This gradual dehydration eliminates the osmotic shock and damage that occur when crystals are directly soaked in high salt or high PEG solutions.

The Crystal Dehydration and Salvage Kit contains the 12 saturated salt solutions listed in Table 1, 20 MicroRT capillaries, and 6 GB-B3S goniometer bases that mate with the capillaries to produce a sealed environment for dehydration. The bases accept SPINE and 19 mm length MicroMounts, MicroLoops, MicroMeshes and MicroGrippers, and 18 mm nylon loops.

The 12 salts produce relative humidities from 97% to 22%, with dense coverage of the range between 93% and 60% of greatest relevance in protein crystallography. The chosen salts have low to moderate toxicity, and produce relative humidities that vary little with temperature near 25°C.

Using the Kit

To dehydrate a crystal, inject 10-20 μl of a salt solution into a MicroRT capillary tube. Use a gel-loading pipette tip to inject the liquid all the way down toward the sealed end of the tube. This will keep the crystal and solution well separated.

Insert a MicroMount, MicroLoop or other mounting loop into a goniometer base and harvest your crystal.

Draw the MicroRT capillary tube over the crystal and onto the goniometer base. A microscope or low-power magnifier can make it easier to guide the capillary tube past the crystal. A small amount of oil or grease applied to the goniometer base can improve the seal to the capillary, but in general is not necessary.

Allow 30 minutes (for crystals 100 μm or smaller) to 2 hours (for ~ 500 μm crystals) for the crystal to equilibrate with the salt solution before X-ray examination.



Figure 1: Usage of the Crystal Dehydration and Salvage Kit

Dehydration Strategies

To quickly determine if there is any relative humidity that yields improved diffraction, choose a salt solution that gives a relatively low final relative humidity – in the 60-75% range. Immediately after enclosing the sample in the MicroRT capillary, place the sample in the X-ray beam and begin acquiring a series of short-exposure frames. Continue until either the unit cell stops changing (indicating that the crystal has equilibrated with the salt solution) or the diffraction degrades dramatically.

For a more systematic study, choose a series of salt solutions covering a range of relative humidities, say from 93% to 68%. Inject the salt solution with the highest relative humidity into a MicroRT capillary, harvest a crystal, and draw the capillary over the crystal and onto the goniometer base. Allow the crystal to equilibrate, and then acquire a few frames of X-ray data, sufficient to determine cell parameters and resolution. Remove the MicroRT capillary, immediately replace it with a capillary containing the solution with the next highest relative humidity, allow the crystal to equilibrate and then take a few more frames. Repeat until you have explored the relative humidity range of interest. Then pop on the capillary with the salt solution that gives the best diffraction, equilibrate, and flash cool to evaluate the low temperature diffraction quality.

Crystal Dehydration and Salvage Kit

In this “serial” approach, the crystal gradually accumulates radiation damage, and this damage may obscure dehydration-induced improvements. Dehydration experiments can also be performed in parallel. Harvest several crystals, and enclose each in a capillary containing a different salt solution. Allow the crystals to equilibrate, and then examine their diffraction to identify the optimum salt / humidity.

Some salt crystals may form around the crystal during dehydration. The diffraction spots or rings from these are easily identified and can usually be eliminated from your analysis.

References

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- [5] Perutz (1954) The Structure of Haemoglobin. III. Direct Determination of the Molecular Transform. *Proc. R. Soc. London Ser. A* **225**:264.
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