

Crystallophores Xo4 are supplied in microtubes having a conical bottom and screw cap under Argon. The aspect of the product can be distorted by an effect of temperature or transport (*white powder to light brown oil*), but it does not change its efficiency.

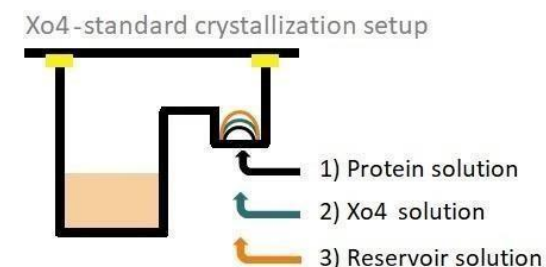
Xo4 as NUCLEATING AGENT

As pre-screening agents, Xo4 lanthanide complexes allow to provide new crystallization conditions, induce alternative crystal packing or improve crystals quality (Engilberge et al, Chem. Science., 2017 & Engilberge et al., Chem. Eur. J., 2018).

	Xo4 product	LuXo4-01	TbXo4-02
<u>Xo4 solution preparation:</u>	Masse for P vials	0.6 mg	0.66 mg
	Volume of milliQ H ₂ O for a 10 mM Xo4 solution	100 µL	100 µL
	Masse for N vials	0.15 mg	0.17 mg
	Volume of milliQ H ₂ O for a 10 mM Xo4 solution	25 µL	25 µL

New Xo4-standard crystallization setup: Each drop is formed by a **3-steps** addition of:

- 1- **1 vol of protein solution**
- 2- **+ 1 vol of Xo4 solution**
- 3- **+ 1 vol of reservoir solution.**



NB: All screens are compatible for the crystallization in the presence of Xo4.

It is recommended to test the crystallization with and without Xo4 using the new Xo4-standard crystallization setup.

It is also possible to make aliquots of Xo4 solutions (to keep at -20°C) and also to adapt the Xo4 concentration:

⇒ If your protein tends to precipitate during screening, you can go down to 2mM; if, on the other hand, it is very stable in its buffer, you can go up to 20mM and more.

Xo4 as PHASING AGENT

Xo4 molecules (lanthanide complexes) are used in structural biology to experimentally solve the phase problem and they are fully compatible with conventional phasing methods (Engilberge et al, J. Appl. Cryst., 2019 & Engilberge et al., Chem. Sci., 2017).

Phasing setup:

- 1- A short centrifugation of the microtube (**P type vials or Large type vials**) allows the powder to form a pellet.
- 2- The pellet is then re-suspended with **10 µl** of mother liquor containing a cryo-protectant to reach a final Xo4 concentration of **100 mM**
- 3- The solution is centrifuged at 10000 rpm for 2 minutes.
- 4- A drop of 1µl is deposited on a coverslip
- 5- Crystals are harvested and soaked in drops containing Xo4 **and** the mother liquor with cryo-protectant (**no back soaking**) (one crystal per drop for an optimal protocol)
- 6- Crystals are flash frozen in liquid nitrogen

NB: Phasing with Xo4 is also recommended with co-crystals obtained with 10mM Xo4 solution.

