

APPLICATION NOTE:

New Xo4 standard crystallization and phasing setup



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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 solution	Product	TbX04-01-N / LuX04-01-N	TbX04-02-N	TbX04-01-P / LuX04-01-P	TbXo4-02-P
<u>preparation:</u>	Mass (mg)	0.15	0.17	0.6	0.66
	Volume of milliQ H ₂ O for a 10 mM Xo4 solution (μ L)	25	25	100	100

<u>New Xo4-standard crystallization setup:</u> 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.**



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 (MDL)) 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.





