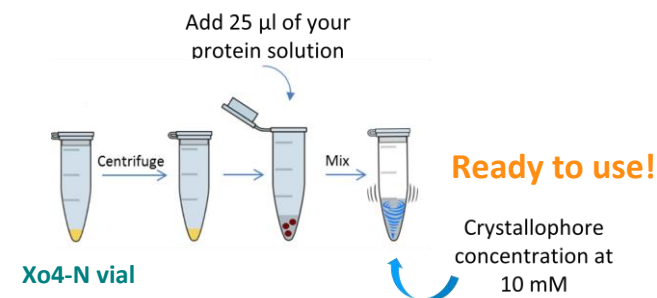


TbXo4-02-N as PRE-SCREENING AGENT (0.17 mg)

TbXo4-02-N provides new crystallization conditions and it also improves the quality of the obtained crystals. Prior to automated crystallization experiments, the TbXo4-02-N shall be directly dissolved with the protein solution at a concentration of 10 mM before running crystallization screening.

Methods of use:

- 1- A short centrifugation of the microtube allows the powder* to form a pellet.
- 2- The pellet is then re-suspended with 25 μ l of protein solution to reach a final TbXo4-02-N concentration of 10 mM.
- 3- Mix it. (The protein solution may turn cloudy without affecting the subsequent screening. Check for nano/micro crystals[▲])
- 4- The solution shall be **immediately** used for crystallization.



[▲]2'- It's also possible to play with the Xo4 concentration, as hits have already been observed from 2 mM and up to 50 mM and more: by forming the drop through the mixing of 1 vol of protein solution + 1 vol of Xo4 solution + 1 vol of precipitant (in that order = Xo4 first). In the case of a cloudy solution after addition of the protein solution, repeat the process by dissolving first the crystallophore powder with a solution of NaHCO₃ 10mM at pH 7

3'- Use it immediately for your screening trials by doing a 1+1+1 drop (**in that order: protein solution + Xo4 solution + precipitant solution**)

Depending on the protein, different screens can be used. In order to get as many exploitable hits as possible, the optimal crystallization experiment would combine screening of conditions of the protein both in native conditions and in presence of TbXo4-02-N (i.e.: two drops shall be made, without and with 10 mM TbXo4-02-N).

TbXo4-02-P as PHASING AGENT (0.66 mg)

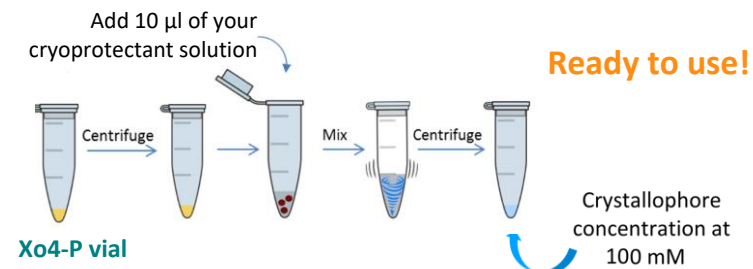
TbXo4-02-P is fully compatible with conventional phasing methods. A major advantage is that it overcomes the tedious and time-consuming work of seleno-methionine labelling or of heavy atom incorporation. Prior to data collection, a rapid soaking of crystals (from 30 sec to 5 min) in a concentrated TbXo4-02-P solution (100mM) is done in their respective mother liquor supplemented with a cryo-protectant.

Methods of use:

- 1- A short centrifugation of the microtube (TbXo4-02-P) allows the powder* to form a pellet.
- 2- The pellet is then re-suspended with 10 μ l of mother liquor containing a cryo-protectant to reach a final TbXo4-02 concentration of 100 mM.
- 3- The solution is centrifuged at 10000 rpm for 2 minutes.
- 4- A drop of 1 μ l is deposited on a coverslip
- 5- Crystals are harvested and soaked in the drop containing TbXo4-02-P **and** the mother liquor with cryo-protectant (**no back soaking**)
- 6- Crystals are flash frozen in liquid nitrogen

Note : We would recommend to soak only one crystal per drop (the concentration in the drop decrease quickly after 1 or 2 soaks).

NB: Phasing with TbXo4-02-P (100mM) is also recommended with co-crystals obtained with 10mM TbXo4-02-N



Use mother liquor or aliquots of Xo4 rapidly and store them frozen at -80°C until further use.

*can also be a light brown oil: the aspect of the product can be distorted by an effect of temperature or transport, but it does not change its efficiency.