

Molecular
Dimensions



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Polyvalan Crystallophore N°1 (Tb-Xo4)

New soluble additive for crystallization screening with all-in-one properties (Nucleant, Luminescent and Phasing)

Available as: Polyvalan Crystallophore N°1 1 x 0.6mg MD2-80

Polyvalan Crystallophore N°1 5 x 0.6mg MD2-81

*Polyvalan Crystallophore N°1 is supplied in a 1.5 ml microtube (conical bottom and a screw cap for inserts with O-Ring). Each microtube contains 0.6 mg of Terbium macrocyclic complex **Tb-Xo4** (Figure 1).*

Description

Crystallophore N°1(Tb-Xo4) – is a unique, patented chemical tool for protein crystallography developed by Polyvalan, Lyon, France.

Tb-Xo4 is a rapid, easy-to-use tool that presents multiple advantages:

- (i) It is soluble and stable in all crystallisation conditions and consequently fully compatible with high throughput screening;
- (ii) It acts as a strong **Nucleating Agent** providing new crystallisation conditions and generally leading to the formation of bigger, **Tb-Xo4** derivatised crystals, ready-to-use for X-ray diffraction;
- (iii) Under UV irradiation, the complex emits in the green (**Luminescent**) enabling very easy detection of the **Tb-Xo4** derivatised crystal in crystallization drops;
- (iv) and finally the crystallophore is a straightforward **Phasing Agent** facilitating crystal structure determination using SAD or MAD methods, due to the exceptional anomalous properties of f-block elements.

Storage

Store powdered **Tb-Xo4** at room temperature (20°C) and protect from moisture. Dry, unopened samples will keep for 1 year at 20°C in dry conditions (the product is very hygroscopic).

Diluted/hydrated **Tb-Xo4** (in water or buffer) will keep for 2 months.

When **Tb-Xo4** has been mixed with protein we recommend using the resulting solution immediately,

Ship

Ambient Temperature Only

Standard Handling

United Kingdom, Europe and Australia

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Polyvalan Crystallophore N°1 (Tb-Xo4)

About 10,000 new proteins structures are obtained each year through the use of synchrotron facilities and automatic crystallization platforms that screen thousands of crystallization mixtures. Despite these large scale efforts, end-user needs are unsatisfied with the existing offering: less than 30% of the proteins that are expressed and purified yield diffraction quality crystals and of these only 67% provide structures (20% of the total expressed and purified samples).

Tb-Xo4 is a Terbium macrocyclic complex (Figure 1), that allows the user to dramatically improve the yield of diffraction quality crystals than by native alone. **Tb-Xo4** is a complementary product and not a substitute for screening with native protein alone.

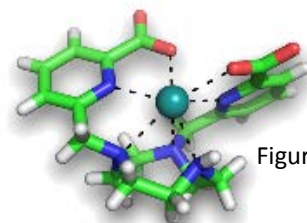


Figure 1

Polyvalan Crystallophore N°1 Tb-Xo4 is a rapid, easy-to-use tool that presents multiple advantages:

- (i) it is soluble and stable and consequently fully compatible with high throughput screening and conventional phasing methods;
- (ii) it acts as a strong nucleating agent providing a 40% (on average) increase in new crystallization conditions and generally leading to the formation of bigger **Tb-Xo4** derivatised crystals, ready-to-use for X-ray diffraction (Figure 2);
- (iii) under UV, the complex emits in the green enabling very easy detection of the Tb-Xo4 derivative crystal in crystallization drops (Figure 3);
- (iv) and finally, it is a straightforward phasing agent that overcomes the tedious and time-consuming work of existing solutions: seleno-methionine labelling or heavy-atom incorporation.

Tb-Xo4 is a **complementary product** and not a substitute for commercial crystallization kits. **Tb-Xo4** should be used as an additive in screening kits and therefore fits with current end users practices. It should be directly dissolved in the protein solution at a concentration of 10 mM before crystallization screening.

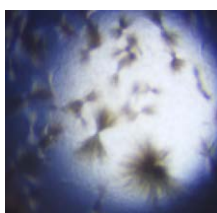


Figure 2. Crystals of pb6 protein from T5 phage: native conditions (on left); with Tb-Xo4 (on right).

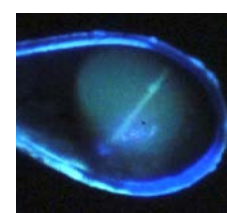


Figure 3. Crystallophore #1 Luminesces strongly in the visible region under UV excitation making detecting crystals in drops (on left) and loops (on right) easy

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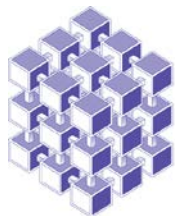
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Poylvalan Crystallophore N°1 User Guide:

Tb-Xo4 is a *complementary product* and not a substitute to commercial crystallization kits.

Use of Tb-Xo4 as NUCLEATING AGENT

Tb-Xo4 not only provides new crystallization conditions but also improves the quality of the crystals obtained.

Prior to automated crystallization experiments, Tb-Xo4 should be dissolved in the protein solution to a concentration of 10 mM.

Method of use:

1. Give the microtube a short centrifuge to allow the powder to form a pellet at the bottom of the tube.
2. Re-suspended the pellet with 100µl protein solution to reach a final **Tb-Xo4** concentration of 10 mM.
3. Centrifuge the solution at 10,000 rpm for 5 minutes to remove possible aggregate.
4. Immediately use the solution for crystallization.

For automated screening trials, the crystallization experiments should be set up by mixing 100nL of protein + **Tb-Xo4** solution with 100nL of precipitant solution (6 standard screens / 576 conditions).

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 the presence of **Tb-Xo4** (i.e. : two drops should be made, with and without 10 mM **Tb-Xo4**).

Use of Tb-Xo4 as PHASING AGENT

Tb-Xo4 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, carry out a rapid (from 30 sec to 5 min) soak of crystals in a concentrated **Tb-Xo4** solution (100mM) in mother liquor supplemented with a cryo-protectant.

Method of use:

1. Give the microtube a short centrifuge to allow the powder to form a pellet at the bottom of the tube.
2. Re-suspended the pellet with 10µl mother liquor or cryo-protective solution to reach a final Tb-Xo4 concentration of 100 mM.
3. Centrifuge the solution at 10000 rpm for 5 minutes.
4. Add the resulting solution to the crystallization drop using a soaking time ranging from 30 sec to 5 mins.
5. Crystals can then be harvested on a single Mesh LithoLoop or ActiLoop.

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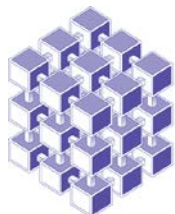
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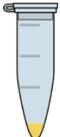
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6. Crystals can then be cryo-cooled in liquid nitrogen.
7. Carry out Data collection.
8. Phasing and model building with e.g. Crank

To fully exploit the anomalous contribution of Terbium, an X-ray fluorescence scan can be performed to precisely define the peak and the inflection wavelengths of the Tb L_{III} absorption peak and to set the energy of the X-ray beam.

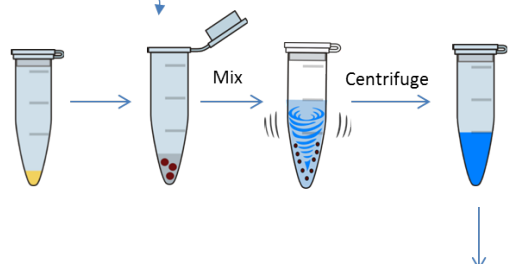
Overview of the protocol for Tb-Xo4

1.  0.6mg of Tb-Xo4

2. Prior to solubilization, centrifuge the powder to form a pellet (~1 min at ~10000rpm)

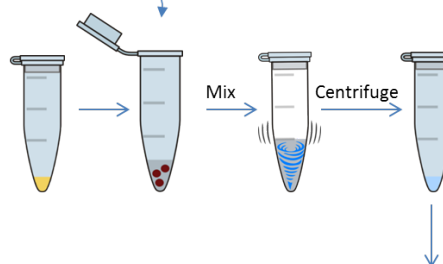
3. Crystallization screening with 10 mM Tb-XO4 (final concentration) or 4. Soaking for phasing (100mM Tb-XO4)

Add 100 μ L of protein solution

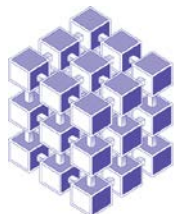


The solution is ready for use.
576 conditions can be screened with one tube
@100 nL per drop.

Add 10 μ L of crystallization condition or cryo protectant solution



The solution is ready for soaking crystals to increase the phasing power.



Tips:

- Excitation possible between 250 and 380 nm.
- Emission is in the visible range between 475 and 635 nm.
- Crystals glow with a green light and are easy to discern 'by eye':
- Tb^{3+} has 62 electrons making it ideal for heavy atom phasing.
- Powerful tool for SAD and SIRAS phasing: Terbium's L_{III} absorption edge is around 1.6 Å (7.5 KeV, $f'' \sim 30$ electrons), with significant anomalous signal present far from the edge.



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