JBS Tantalum Cluster Derivatization Kit

Phasing Kit

Cat. No.	Amount
PK-103	6 x 1 mg

For *in vitro* use only
Quality guaranteed for 12 months
Store at room temperature under an argon atmosphere. Once the vials have been opened floating with argon is recommended for long term storage.

Application

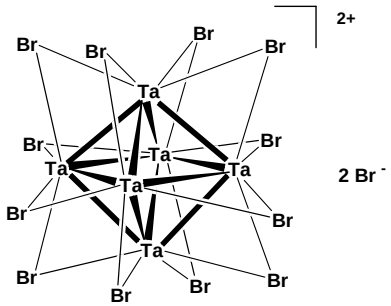
Heavy atom derivatization of biological macromolecules for isomorphous and/or anomalous phasing methods.

Kit Contents

6 pre-weighted solid aliquots of hexatantalum tetradecabromide at 1 mg.

Specifications

Name: Hexatantalum tetradecabromide
Formula: $[Ta_6Br_{12}]^{2+} \times 2\ Br^-$
MW: 2204,3 g/mol
Appearance: green powder
Water Solubility: ~ 2 mM
Manufacturer: proteros



Features

The Tantalum Bromide Cluster is used for the preparation of heavy-atom derivatives for structure determination of biological macromolecules by X-ray analysis. Electron-rich compounds are utilized not only in single and multiple isomorphous replacement (SIR and MIR), but also in single and multiple anomalous diffraction (SAD and MAD) experiments. They induce measurable changes in the diffraction intensities allowing phase calculation required for structure determination.

The hexatantalum bromide cluster is built of an octahedron of 6 metal atoms with 12 bridging bromine atoms along the 12 edges of the octahedron. The cluster is highly symmetrical and has a radius of approximately 4.3 Å.

The two present anomalous scatterers Ta and Br display different absorption edges, useful for determining the cluster orientation for low resolution datasets [1]:
Ta L-III 9.8811 keV (1.2548 Å)
Br K 13.4737 keV (0.9202 Å)

Tantalum Bromide Clusters have been successfully employed in several structural studies because of their high electron-density, solubility in aqueous solutions and stability over a wide pH range [1-4].

Usage

Heavy-atom derivatization is performed by either:

- Soaking protein crystals with a soaking solution containing the Tantalum Bromide Cluster.

  or

- Adding Tantalum Bromide Cluster directly to the crystallization drop containing the crystals.

Soaking

For soaking experiments, protein crystals are transferred into a stabilizing solution drop containing the crystallization solution together with the heavy atom compound. Make sure that the stabilizing solution contains all the components of the precipitant solution in which the protein was crystallized, otherwise the crystal could start to suffer upon soaking. Concentration of Ta_6Br_{12} in the final soaking solution and soaking time is dependent on the protein under investigation. However, it is usually recommended to use a high concentration of the heavy atom compound in conjunction with a short soaking time. Concentrations of Ta_6Br_{12} in soak

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solutions have been in the range of 1 mM up to saturation. If crystal degradation like cracking or dissolution occurs, decreasing compound concentration and extending soaking incubation time, along with a gradient soak, may be advised. Crystal cross-linking is also helpful to stabilize crystal lattice when the soaking approach is applied [5].

(1) Add 227 µl of deionized water to the pre-weighted solid aliquot of Ta_6Br_{12} and mix well in order to prepare a 2 mM stock solution of Ta_6Br_{12}. This stock solution is stable at -20°C for 2 weeks.

Please note: It is possible to obtain a stock solution of Ta_6Br_{12} directly from the mother liquor where crystals have grown. Just have in mind that the solubility will be dependent on the condition in use. In this case, add 227 µl of mother liquor directly to the pre-weighted solid aliquot of Ta_6Br_{12} in order to obtain a 2 mM soaking solution. Place a drop of 2 µl on the top of a cover slide and proceed directly to step 4.

(2) Prepare a stabilizing solution, oriented on your crystallization condition, with a volume of e.g. 10 ml.

Please note: Soaking is usually performed in a stabilizing solution, wherein the protein crystal is stable. The concentration of each component from the stabilizing solution has to be determined experimentally. As starting point we recommend the same FINAL concentrations (after addition of the heavy atom compound) as the reservoir solution where crystallization occurred. Hypotonic shocks (when concentration of stabilizing solution is lower than crystallization solution) can lead to irreversible crystal damage/dissolution while mild hypertonic shocks can cause crystal dehydration, often used to improve diffraction data [6, 7].

(3) Prepare 2 µl of the soaking solution on a cover slide by mixing the stabilizing solution (without Ta_6Br_{12}) with the stock solution of Ta_6Br_{12} at the desired ratio, e.g. for a 1 mM Ta_6Br_{12} soaking solution, mix 1 µl Ta_6Br_{12} (2 mM) + 1 µl stabilizing solution. **Remember that in this case your stabilizing solution should be 2x the desired final concentration.**

(4) Transfer the crystal into the soaking solution using a loop, or a MicroMount™ and place the cover slide on the top of a well containing the original crystallization condition.

(5) Observe the crystal under a microscope to check for degradation. Crystals should become green while the green color of the soaking solution fades after some days of incubation (1-2 days). Afterwards, proceed with usual crystal mounting for X-ray data collection.

It may be advised to test the soaking conditions with a low quality crystal and if no problems occur then proceed with a similar but high quality crystal.

Due to the relative low solubility of Tantalum Bromide Cluster in water, successful derivation has been also reported by adding solid Ta_6Br_{12} directly to the crystallization drop. For this purpose, Grasp a few crumbs of the solid Tantalum Bromide Cluster with a micro-spatula and add them to the crystallization drop containing pre-grown protein crystals. The metal cluster will dissolve within a few hours and the protein crystals will turn green.

Phasing

At low resolution (> 6 Å) it can be assumed that all atoms of the cluster scatter in phase. Therefore, the cluster can be considered as a "super heavy atom", easily to locate in difference Patterson maps. At high resolution the individual atoms of the cluster can be used for phasing after positioning the cluster in the unit cell [1, 8].

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Safety Information

Heavy atom compounds are toxic substances and should be treated as such.

Work carefully and clean when handling heavy atom compounds.

Wear gloves when handling heavy atom reagents. Wear gloves when handling crystals and any laboratory material that was in contact with heavy atom compounds.

Keep in mind that all glassware and laboratory material (such as spatulas etc.) that have been in contact with heavy atom compounds must be considered as contaminated and should be handled accordingly.

Label all plates and lab ware containing heavy atom compounds appropriately.

Get to know the appropriate hazardous material guidelines for handling and disposal of heavy atom compounds, heavy atom reagents, and laboratory equipment contaminated with heavy atoms.

References

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[4] Szczepanowski *et al.* (2005) Crystal structure of a fragment of mouse ubiquitin-activating enzyme. *J. Biol. Chem.* **280**:22006.

[5] Begona *et al.* (2005) Post-crystallization treatments for improving diffraction quality of protein crystals. *Acta Cryst.* D**61**:1173.

[6] Lopez-Jaramillo *et al.* (2002) Soaking: the effect of osmotic shock on tetragonal lysozyme crystals. *Acta Cryst.* D**58**:209.

[7] Garman *et al.* (2003) Heavy-atom derivatization. *Acta Cryst.* D**59**:1903.

[8] Dahms *et al.* (2013) Localization and orientation of heavy-atom cluster compounds in protein crystals using molecular replacement. *Acta Cryst. D***69**:284.

Selected Literature Citations

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- De *et al.* (2011) Crystal structure of the *Vibrio cholerae* cytolysin heptamer reveals common features among disparate pore-forming toxins. *PNAS* **108(18)**:7385.