JBS Paratungstate Cluster Kit

Phasing Kit

Cat. No.	Amount
PK-107	6 x 3.5 mg

For *in vitro* use only.
Quality guaranteed for12 months.
Store at room temperature.

Application

Heavy atom derivatization of biological macro-molecules for isomorphous and/or anomalous phasing methods.

Kit Contents

6 pre-weighted solid aliquots of Deca-Ammonium-paratungstate at 3.5 mg.

Specifications

Name: Deca-Ammonium paratungstate
Formula: $(NH_4)_{10}[H_2W_{12}O_{42}] \times H_2O$
MW (anhydrous): 3060 g/mol
Appearance: white crystalline powder
Water Solubility: 0.1 g/l (33 µM)
Stability: stable in acidic and neutral solutions at pH 5 - 8. The cluster undergoes hydrolysis to monomeric WO_4^{2-} units in basic solutions (pH > 8).

Features

The structure of the paratungstate cluster is composed of 12 tungsten(VI) metal centers, each coordinated octahedrally by six oxo O^{2-}ligands. Neighbouring tungsten centers are linked to one another through bridging, adjacent oxo ligands. There are a total of 28 bridging oxygen atoms that link the 12 tungsten centres. The metal centres in the 12 octahedra are arranged in an ellipsoidal cluster where two trigonal W_3O_{13} units are linked by two peripheral $[W_3O_{11}]$ chains. The bond length from the central oxygen to the tungsten atoms is ca. 2.35 Å. The bond length between the tungsten atoms and each of the bridging oxygens is ca. 1.9 Å. The remaining 14 oxygen atoms that are forming W=O double bonds have bond lengths of 1.70 Å. The octahedra are therefore distorted. In the center of the cluster there is an ellipsoidal "void" which contains two protons, H^+. These protons are structurally necessary and cannot be removed from the cluster. As a result, the cluster carries a negative charge of 10-.

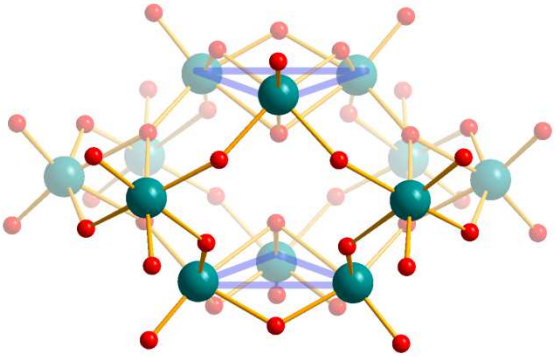

Fig. 1: Ball-and-stick representation of the $[H_2W_{12}O_{42}]^{10-}$-anion. Colour scheme: Tungsten (W) green, oxygen (O) red.

Polyoxotungstate clusters have been successfully employed in structural studies because of their high electron-density and good solubility in aqueous solutions [1].

Tungsten is a strong anomalous scatterer with the L-absorption edges in the energy range of interest (Table 1).

L-I	12.0998 keV	1.0247 Å
L-II	11.5440 keV	1.0740 Å
L-III	10.2068 keV	1.2147 Å

Table 1. Absorption edges of Tungsten

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Usage

Heavy-atom derivatization is performed by soaking protein crystals with a soaking solution containing the paratungstate cluster.

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 $(NH_4)_{10}[H_2W_{12}O_{42}]$ 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 $(NH_4)_{10}[H_2W_{12}O_{42}]$ in soak solutions have been in the range of 1 - 5 mM, although the solubility of Deca-Ammonium paratungstate in water is only 0.1 g/l (33 µM). 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 [2].

(1) Add 119 µl of deionized water to the pre-weighted solid aliquot of $(NH_4)_{10}[H_2W_{12}O_{42}]$ and mix well in order to prepare a 10 mM stock solution of $(NH_4)_{10}[H_2W_{12}O_{42}]$. This stock solution is stable at 4°C for 2 weeks.

Please note: It is possible to obtain a stock solution of the paratungstate cluster directly from the mother liquor where crystals have grown. Just have in mind that stability and solubility will be dependent on the condition in use. In this case, add 238 µl of mother liquor directly to the pre-weighted solid aliquot of $(NH_4)_{10}[H_2W_{12}O_{42}]$ in order to obtain a 5 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 [3, 4].

(3) Prepare 2 µl of the soaking solution on a cover slide by mixing the stabilizing solution (without $(NH_4)_{10}[H_2W_{12}O_{42}]$) with the stock solution of the paratungstate cluster at the desired ratio, e.g. for a 5 mM $(NH_4)_{10}[H_2W_{12}O_{42}]$ soaking solution, mix 1 µl $(NH_4)_{10}[H_2W_{12}O_{42}]$ (10 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 and incubate for 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.

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 the 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].

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

[1] Rudenko *et al.* (2003) 'MAD'ly phasing the extracellular domain of the LDL receptor: a medium-sized protein, large tungsten clusters and multiple non-isomorphous crystals. *Acta Cryst.* D**59**:1978.
[2] Begona *et al.* (2005) Post-crystallization treatments for improving diffraction quality of protein crystals. *Acta Cryst.* D**61**:1173.
[3] Lopez-Jaramillo *et al.* (2002). Soaking: the effect of osmotic shock on tetragonal lysozyme crystals. *Acta Cryst.* D**58**:209.
[4] Garman *et al.* (2003). Heavy-atom derivatization. *Acta Cryst.* D**59**:1903.