DATA SHEET

JBS Halo-ATP Kit

Cat. No.	Amount
PK-101	1 Kit

For *in vitro* use only
Quality guaranteed for 12 months
Store at -20°C

Application

Incorporation of halogenated ATP analogs into nucleotide binding enzymes for isomorphous/ anomalous phasing.

Kit Contents

The **JBS Halo-ATP Kit** contains 12 halogenated nucleotide analogs as lyophilized sodium salt, with an amount equal to 50 units (1 unit = 1 µl of a 10 mM solution) each.

- 2'-Iodo-ADP
- 2'-Iodo-ATP
- 2'-Iodo-AppNHp (2'-Iodo-AMPPNP)
- 2'-Bromo-ADP
- 2'-Bromo-ATP
- 2'-Bromo-AppNHp (2'-Bromo-AMPPNP)
- 8-Iodo-ADP
- 8-Iodo-ATP
- 8-Iodo-AppNHp (8-Iodo-AMPPNP)
- 8-Bromo-ADP
- 8-Bromo-ATP
- 8-Bromo-AppNHp (8-Bromo-AMPPNP)

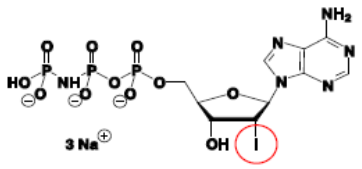
Features

Halogenated nucleotide analogs provide a straightforward method that allows rational incorporation of heavy atoms into a large number of nucleotide-binding enzymes.

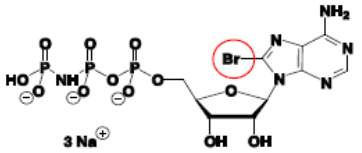
ADP, ATP and the non-hydrolysable analog AppNHp (AMPPNP) are brominated or iodinated at either the 8- or the 2'-position.

Bromine, with an K absorption edge of 13.4737 keV (0.9202 Å) can be used for MAD phasing and iodine is widely used for MIR.

- **2'I-AppNHp**, *as an example for modification of the **2'**-position*



- **8Br-AppNHp**, *as an example for modification of the **8**-position*



It is important to keep in mind that the affinity of the phasing analogs to the protein may differ from that of non-substituted ATP-derivatives [2]. One protein may tolerate a substitution at the sugar (2') but may only weakly bind to base- (8-) substituted analogs or vice versa. Also, an excess of analog may be required in order to completely displace the bound natural ATP derivative.

Since proteins in complex with ADP, ATP or AppNHp tend to show different crystallization behavior, the same may apply to proteins in complex with the respective phasing analogs. Therefore, it is worth trying the whole set of analogs as well as a range of different concentrations.

Usage

Crystal soaking as well as co-crystallization can be used to find the best binder and the highest quality crystals - from only one single 24 well tray.

Crystal soaking is the most straightforward and recommended method if you already have crystals of your protein in complex with an Adenosine nucleotide:

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- dissolve the halogenated analog in deionized water to a concentration that is ≥10x of your desired final concentration in the crystallization drop (Naber et al. [1] recommend 5 mM)
- add ≤1/10 of the crystallization drop volume of the freshly prepared solution into the drop containing your crystals
- depending on the stability of your crystals, incubate for one hour up to several weeks to allow the displacement reaction to take place

In case the crystals get damaged or dissolve upon addition of the phasing analog, dissolve the analog in mother liquor (instead of dissolving it in deionized water, if at all possible), and/or reduce the amount of analog added until the crystals are stable.

If crystal soaking fails to give satisfactory results then *co-crystallization* is recommended. In this case, simply substitute the ATP-derivative used in your crystal setup with the phasing-analogs.

References

[1] Naber *et al.* (1995) A novel adenosine-triphos-phate analog with a heavy-atom to target the nucleotide-binding site of proteins. *Protein Sci.* **4**:1824.

[2] Gruen *et al.* (1999) 2'-Halo-ATP and –GTP analogues: Rational phasing tools for protein crystallography. *Protein Sci.* **8**:2524.