JBS Halo-ATP Kit

The **JBS Halo-ATP Kit** is a Phasing Kit for ATP-binding Proteins.

The **JBS Halo-ATP Kit** consists of 12 analogs of ADP / ATP, and of the non-hydrolysable AppNHp (AMPPNP), brominated or iodinated at the 8- or 2'-position:

- 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)

For the solution of the macromolecular phase problem, the most commonly used methods involve the incorporation of heavy atoms into protein crystals. Finding such derivatives is the second bottle neck in the determination of the 3D structure of biological macromolecules. Most labeling procedures focus on the protein itself in a "trial and error" fashion. Halogenated nucleotide analogs however, provide an alternative method that allows rational incorporation of heavy atoms into a large number of nucleotide-binding enzymes.

The natural affinity of these nucleotides to their binding proteins incorporates the heavy atom into the active site of the protein. Bromine can be used for MAD phasing and iodine is widely used for MIR. Co-crystallization as well as crystal soaking can be used to find the best binder and the highest quality crystals - from only one single 24 well tray.

Crystal soaking

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

- dissolve the halogenated analog in deionized water to a concentration that is ≥ 10x of your desired final concentration in the drop (Naber *et al.* [1] recommend 5 mM)
- add ≤ 1/10 of drop volume into the drop containing your crystal(s) and mix carefully or leave without mixing
- depending on the stability of your crystals, incubate for one hour up to several weeks that the displacement reaction can take place
- fish the crystals and carry on as usual

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.

Co-crystallization

If crystal soaking fails to give satisfactory results then co-crystallization is recommended. In this case, simply substitute the ATP-derivative that you use in your crystal setup with the phasing-analog(s).

IMPORTANT NOTE:

Keep in mind that the affinity of the phasing analogs to your 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. Further, since ADP, ATP and AppNHp usually show different behavior in crystallization experiments, the same may apply to the respective phasing analogs. Therefore, it is worth trying the whole set of analogs as well as a range of concentrations.

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.

Storage and Expiry

Short term exposure (up to 1 week cumulative) to ambient temperature possible. Long term storage at ≤ -20°C. If stored as recommended, Jena Bioscience guarantees optimal performance of this product for 12 months after date of delivery.