

## JBS Halo-GTP Kit

Cat. No.	Amount
PK-102	1 Kit

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

### Application

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

### Kit Contents

The **JBS Halo-GTP Kit** contains 6 halogenated nucleotide analogs as lyophilized sodium salt, with an amount equal to 50 units (1 unit = 1 µl of a 10 mM solution) each.

- 8-Iodo-GDP
- 8-Iodo-GTP
- 8-Iodo-GppNHp (8-Iodo-GMPPNP)
- 8-Bromo-GDP
- 8-Bromo-GTP
- 8-Bromo-GppNHp (8-Bromo-GMPPNP)

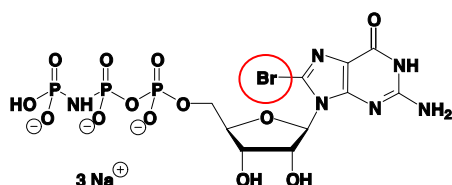
### Features

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

GDP, GTP and the non-hydrolysable analog AppNHp (AMPPNP) are brominated or iodinated at the 8-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.

- **8-Bromo-GppNHp**, as an example for the incorporation of bromine at 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 GTP-derivatives [2]. Also, an excess of analog may be required in order to completely displace the bound natural GTP derivative.

Since proteins in complex with GDP, GTP or GppNHp 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 Guanosine nucleotide:

- dissolve the halogenated analog in deionized water to a concentration that is  $\geq 10x$  of your desired final concentration in the crystallization drop (Naber et al. [1] recommend 5 mM)
- add  $\leq 1/10$  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.

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### References

[1] Naber *et al.* (1995) A novel adenosine-triphosphate 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.