NatX-ray
Advanced Solutions for X-ray Crystallography

Product reference: CSM002-01xxA

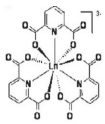
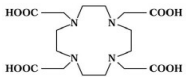
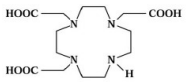
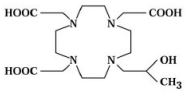
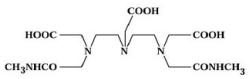
“Scale-up” lanthanide phasing compounds
User Documentation

Please, read carefully this documentation before using. This documentation is also available on www.natx-ray.com

This product contains one lanthanide complex out of the 10 available in the phasing kit (ref.: CSM002-0001A). These compounds are designed for anomalous phasing of protein structures. These molecules contain lanthanide atoms and bind to the surface of macromolecules. With the present compounds, lanthanide derivatives crystals of biological macromolecules are obtained either by soaking or by co-crystallization. The derivative crystals can be used to solve the structure of biological macromolecules by methods taking advantage of the strong anomalous signal of the lanthanides in their L_{III} absorption edge or, for some of them, with CuKα radiation from a laboratory X-ray source.

Kit contents

The 10 complexes available, obtained from five different chelators, are:

Acronym	Name	Formula
$(DPA)_3$	tris(pyridine-2,6-dicarboxylate) or tris(dipicolinate)	
DOTA	1,4,7,10-tetraazacyclododecan-1,4,7,10-tetraacetic acid (CAS # 60239-18-1)	
DO3A	1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid	
HPDO3A	10-(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecan-1,4,7-triacetic acid	
DTPA-BMA	N,N-bis[2-[(carboxymethyl)[(methylcarbamoil)methyl]amino]ethyl]glycine	

Each of these chelators being provided with two different lanthanide atoms:

Atom	LIII edge	f'' at L_{III} edge (e-)	f'' at 1.0 Å (e-)	f'' at 1.54 Å (e-)
Eu	1.7761 Å / 6980 eV	28-30	6.52	**11.26**
Yb	1.3862 Å / 8944 eV	28-30	**9.69**	4.43

These compounds are proposed in alicots numbered as following:

1: Eu-$(DPA)_3$	**3**: Eu-DOTA	**5**: Eu-DO3A	**7**: Eu-HPDO3A	**9**: Eu-DTPA-BMA
2: Yb-$(DPA)_3$	**4**: Yb-DOTA	**6**: Yb-DO3A	**8**: Yb-HPDO3A	**10**: Yb-DTPA-BMA

Each product contains one of these compounds, designed under the reference CSM002-01xxA, where xx is the number present on the label. Compounds are solubilized in deionized water (18.2 MΩ). Each solution is filtered (0.45 µm cutoff).

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Each compound being prepared the following way:

code	chelator	lanthanide atom	MW (gr)	charge	concentration (mM)	volume (µL)
1	$(DPA)_3$	Eu	788.3	3-	200	1000
2		Yb	809.4	3-	200	1000
3	DOTA	Eu	575.3	1-	500	200
4		Yb	596.4	1-	500	200
5	DO3A	Eu	495.3	0	500	200
6		Yb	516.4	0	500	200
7	HPDO3A	Eu	553.0	0	500	200
8		Yb	574.1	0	500	200
9	DTPA-BMA	Eu	568.4	0	500	200
10		Yb	589.5	0	500	200

Usage

$(DPA)_3$
This compound should be used at typical concentrations of 50-100 mM as an additive to the crystallization conditions (co-crystallization or soaking). The compound crystallizes in the presence of divalent ions (Ca^{2+}, Mg^{2+}…) and in high concentration salt conditions. The ionic strength of the compound is high, which strongly modifies the crystallization conditions. Adding the compound to the protein solution may lead to a precipitate from which derivative crystals may grow.
For experiments at the L_{III} absorption edge, the crystal should be washed shortly in a cryo-solution without compound to avoid x-ray fluorescence from the solution surrounding the crystal.
Depending on the crystallization conditions used, the lanthanide atom may be released, and thus the compound has to be considered as a lanthanide-containing compound during manipulation and waste processing. As a consequence, special care has to be made for the manipulation of this compound (use of appropriate personal protective equipment like gloves and glasses). See also safety documentation on www.natx-ray.com.

DOTA, DO3A, HP-DO3A and DTPA-BMA
Unlike chemicals that are commonly used to prepare heavy-atom derivative crystals of biological macromolecules, most of these compounds are not disruptive. As such, it should be used at the highest possible concentration (100 mM or higher) as an additive to the crystallization conditions (co-crystallization) or to the mother liquor (soaking). Soaking time can be rather short (a few 10 s). If for cryo-protection long soaking times in the cryoprotectant are required, then the compound should be introduced at the highest possible concentration into the cryoprotectant. This may be the easiest way for preparing derivative crystals by soaking.
For experiments at the L_{III} absorption edge, the crystal should be washed very shortly in a cryo-solution without compound to avoid x-ray fluorescence from the solution surrounding the crystal.
Under standard conditions used for crystallizing biological macromolecules these compound are extremely stable and can thus be used as standard additives. Nevertheless, since they contain a lanthanide atom, they should be considered as lanthanide-containing compounds during waste processing. See also safety documentation on www.natx-ray.com.

Storage

$(DPA)_3$ has to be stored at room temperature. All other compounds can be stored at room temperature or at 4°C.
Protect from light. Best if used within 12 months of receipt.

Acknowledgment

Authors publishing results thanks to these compounds are welcome to cite the following papers:

For tris(dipicolinate)-lanthanide complexes ($(DPA)_3$ complexes):
Pompidor G, D'Aleo A, Vicat J, Toupet L, Giraud N, Kahn R, Maury O, Angew Chem Int Ed Engl. 47 (2008), 3388-3391.

For the other complexes:
Girard E, Stelter M, Vicat J, Kahn R, Acta Cryst. D59 (2003), 1914-1922.