The CALIXAR™ v2.0 Additive Kit MD1-95

New and improved*[1] Calixarene additives for crystallization of functional Membrane Proteins.

MD1- 95 is presented as a 24 x 50 µL microfuge tubes.

Features of CALIXAR™v2.0 Additive Kit

- Crystallization of functional membrane proteins.
- Suitable for both membrane and soluble proteins.
- Promote crystallization of membrane proteins.
- CALIXAR derivatives generate a network of salt bridges around the protein make hydrophobic contacts and «π-stacking» interactions.
- CALIXAR derivatives enhance protein/ protein interactions and therefore facilitate crystallogenesis
- Overcome the defects, drawbacks and obstacles of prior art- such as detergent-based additive kits and small molecule 'bullet' screens.

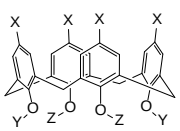
Introduction

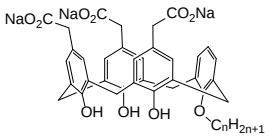
Membrane protein crystallization is notoriously difficult to achieve, being constrained by two hardly-compatible principles: the need of lipids for structuring the membrane domain, and the imperfect folding of proteins outside this specific environment. Amphiphilic molecules such as detergents tend to be used to make the link between these constraints, replacing lipids around the membrane through hydrophobic interactions and maintaining the protein in Protein/Detergent Complexes (PDC). Detergents solubilize the protein in PDC thanks to interactions between transmembrane domains and hydrophobic tails in addition to the exposure of the hydrophilic groups with aqueous media.

Unfortunately, detergents display poor structuring properties – they mask the hydrophobic region of the membrane protein preventing the hydrophilic regions from creating the protein/protein contacts that are necessary for the formation and stabilization of crystal. *The* **CALIXAR™ v2.0 Additive Kit** *promotes the crystallization of membrane proteins. Crystallization is improved by means of the formation of an organized aggregation state or 'supramolecular lumps' of the polar and/or positively charged molecules in space, leading to the formation of crystals.*

The ***CALIXAR™ Additive Kit v2.0 (MD1-95)*** *has the feature of facilitating crystallization through the ability of* CALIXAR derivatives *to induce 'supra-molecular clusters' of micellar-type and to facilitate protein/ protein interactions.*

CALIXAR derivatives have been designed & synthetized for their capacity to generate a network of salt bridges around the protein, in close proximity to the membrane domain with positively-charged residues located at the membrane-cytosol interface of the protein.





CALX004PHO : X = -$CH_2PO(OH)(ONa)$; Y = Z = -H
CALX2PHO0 : X = Z = -H ; Y = -PO(OH)(ONa)
CALX004SFO : X -SO_3Na ; Y = Z = -H
CALX4ACE0 : X = -H ; Y = Z = -CH_2CO_2Na

CALX113ACE : n = 1
CALX153ACE : n = 5

Figure 1 shows the chemical structure of the **CALIXAR derivatives**.

[1] *The calixarenes in this kit have been updated and replace the old CALIXAR Kit MD1-80. These are still CALIXAR derivatives and all that has changed are the names and concentrations.*

CALIXAR additives in membrane protein crystallization.

The new **CALIXAR** derivatives used in this additive kit are ionic molecules with polar groups grafted onto the upper or lower rim of the calix-[4]-arene:

CALX2PHO0 (two phosphonate groups on lower rim), **CALX004PHO** (four phosphonate groups on upper rim), **CALX004SFO** (four sulfonate groups on upper rim), **CALX4ACE0** (four carboxylate groups on lower rim).

CALX113ACE and **CALX153ACE** behave as surfactants, forming micelles of 2-5 nm (Figure 2), with the critical micellar concentration (CMC) being as expected sensitive to pH and ranging from 7.5 to 12.5 mM.

Three aromatic rings are substituted onto upper rim with three hydrophilic sodium carboxylate groups, $-CH_2CO_2Na$, at the para position of aromatic rings of the calix-[4]-arene scaffold. The hydrophobic part is composed by one methyl (**CALX113ACE**) or pentyl (**CALX153ACE**) grafted onto one phenolic group.

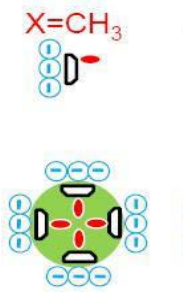


Figure 2 : CALX113ACE

'Supramolecular lumps'- molecular glue.

Supramolecular lumps are the organization of the previous **CALIXAR** derivatives with micellar-type behaviour typically **CALX113ACE** and **CALX153ACE**. These 'supramolecular lumps' become intercalated between the membrane proteins via ionic interactions (Figure 3). These interactions make it possible to reinforce the cohesion of the edifice and facilitate its crystalline organization. In particular, **CALIXAR** derivatives become organised so as to generate 'surpamolecular lumps' that expose on their surface negative charges of their carboxylate functions, maintained by the hydrophobic region of the aliphatic chains.

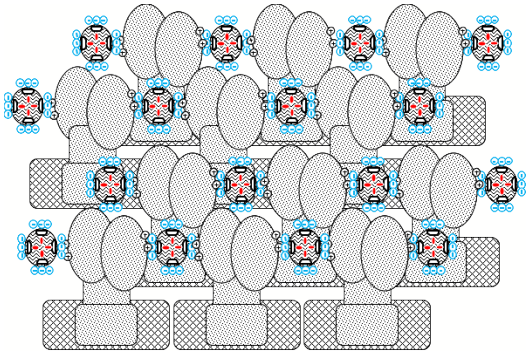


Figure 3

Crystals of a 12TMD protein grown in the presence of **CALIXAR** derivatives.

Instructions for Use:

For a typical 24-well experiment:
Add 2 µL of membrane protein sample, 0.4 µL of the **CALIXAR** derivatives additive and 1.6 µL of a crystallization screen. The reservoir is filled with 500 µL of the screen solution.

Suggested working pH is between 7.5 and 14 to maintain solubility. To use at more acidic pH, the **CALIXAR** derivative will have to be used at a higher dilution than suggested.

CALX2PHO0 is insoluble in water and needs to be used in solution with DMSO.

Recommended storage for the CALIXAR Additive Kit is 4°C. (CALIXAR derivatives can be frozen at -20 ° C and heated up to 80 ° C).

Formulation Notes:

CALIXAR v2.0 Additive Kit reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the MSDS. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.
Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

Manufacturer's safety data sheets are available to download from our website.

References:

1. Protein crystallization additives, use and process". Falson, P. *et al*. - WO 2010116055, Matar-Merheb R, Rhimi M, Leydier A, Huché F, Galián C, Desuzinges-Mandon E, Ficheux D, Flot D, Aghajari N, Kahn R, Di Pietro A, Jault JM, Coleman AW, Falson P. PLoS One. **2011** Mar 31;6(3):e18036
2. Protein camouflage in cytochrome c–calixarene complexes, McGovern, R. E., Fernandes, H Khan, A. R., Power, N. P. & Crowley, P. B. *Nat Chem* **4,** 527–533 (**2012**)

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Table 1. CALIXAR additives are presented as 4 different concentrations per compound.

The CALIXAR v2.0 Additive Kit, mM (final concentrations)					
CALX004PHO	**CALX113ACE**	**CALX153ACE**	**CALX4ACE0**	**CALX004SFO**	**CALX2PHO0**
0.5	0.5	0.5	0.5	0.5	0.5
1	1	1	1	1	1
5	5	5	5	5	5
10	10	10	10	10	10

	CALX004PHO	**CALX113ACE**	**CALX153ACE**	**CALX4ACE0**	**CALX004SFO**	**CALX2PHO0**
MW (g/mol)	888.45	678.57	734.67	744.57	832.65	628.42
CMC, water, mM	-	12.5±0.5	7.5±1.5	-	-	-

Table 2: Molecular weights and critical micellar concentration (CMC) of the CALIXAR derivatives used in The CALIXAR™ v2.0 Additive Kit.

Manufacturer's safety data sheets are available from our website.

Ordering Details:

Catalogue Description	**Catalogue Code**
The CALIXAR™ v2.0 Additive Kit	MD1-95
The CALIXAR™ v 2.0 Additive Single Reagents (50 µL)	MDSR-95-tube number