

The CALIXAR™ C2B Kit

MD1-109

Increase your protein stability and grow better diffracting crystals

MD1-109 is presented as a 10 x 50 µL microfuge tubes
(3 x calixarene compounds, 6 x fluorinated and 1 x hydrogenated surfactants)

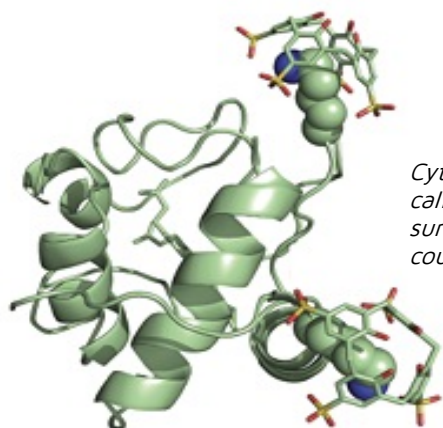


Features of The CALIXAR™ C2B Kit:

- Proven to increase both stability in solution and crystallogenesis of membrane proteins.
- Promotes crystallization of functionally active membrane proteins.
- Suitable for both membrane and soluble proteins.
- Includes six fluorinated stabilising surfactants and one hydrogenated surfactant.

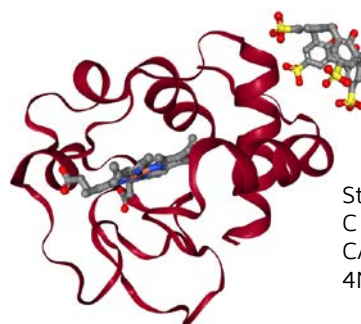
This exciting new kit provides two varieties of stabilizing surfactants: Calixarenes¹⁻⁵ and fluorinated poly(tris)⁶⁻⁸ and bis-glucose⁹⁻¹¹ surfactants. This new kit has been developed by CALIXAR SAS in partnership with the joint laboratory Chem2staB (C2B) at Avignon University, France.

CALIXAR C2B derivatives have been designed & synthesized 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. They can also form a 'cage' around surface lysines, creating a 'charge shield'.



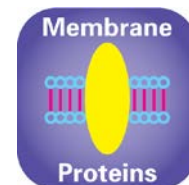
Cytochrome C with calixarenes caging two surface lysines. Image courtesy of P. Crowley

"The CALIXAR™ C2B Kit (MD1-109) has the feature of facilitating crystallization through the ability of CALIXAR derivatives and LC-fluorinated surfactant-type molecules to induce 'supra-molecular clusters' of micellar-type and to facilitate protein/protein interactions."



Structure of Cytochrome C with bound CALX004SFO (PDBID: 4NOK)

Fluorinated surfactants act in a very different manner to their hydrogenated equivalents due to the increased size of fluorine relative to hydrogen and its highly hydrophobic nature. Fluorinated surfactants are unable to extract proteins from the membrane, but can be useful in subsequent purification steps as they do not strip natural lipids and other co-factors from the proteins. In addition, the bulky fluorinated tails cannot penetrate into the interior and disrupt structure. Fluorinated surfactants often decrease non-specific aggregation and are thought to result in improved distribution on cryo-EM grids and better vitrification for cryo-EM data collection. They are also reported to increase crystallizability.



Instructions for Use:

For a typical 24-well experiment:
Add 2 μL of membrane protein sample, 0.4 μL of the **CALIXAR C2B** 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 6-9 to maintain solubility. To use at more acidic pH, the **CALIXAR C2B** derivative will have to be used at a higher dilution than suggested.

Recommended storage for the CALIXAR C2B Kit is 4°C. (CALIXAR C2B derivatives can be frozen at -20 ° C and heated up to 80 ° C).

Formulation Notes:

The CALIXAR C2B Kit reagents are formulated using ultrapure water (>18.0 M Ω). No preservatives are added.

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

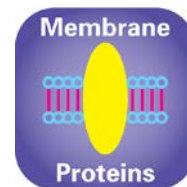
Calixarenes 1) Matar-Merheb, R et al. PLoS One 31: e18036 (2011). 2) McGovern RE et al. Nat Chem 4: 527 (2012). 3) McGovern et al. Chem. Commun.50, 10412-10415 (2014). 4) McGovern et al. Chem. Sci., 6, 442-449 (2015). 5) Rennie, ML et al. Angew. Chem. Int. Ed. Engl. 56: 5517-5521 (2017).

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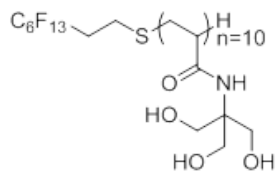
Hydrogenated and Fluorinated surfactants with bis-glucose headgroup 9) Abła M et al. J Colloid Interface Sci 445: 127 (2015).10) Abła M et al. J Org Chem 73: 8142 (2008). 11) Breyton C et al. J Biol Chem 288: 30763 (2013).12) Abła M et al. J Fluorine Chem 134: 63 (2012).



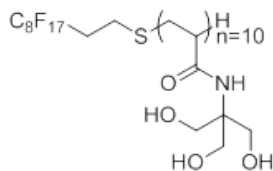
Crystals of membrane proteins grown in the presence of **CALIXAR C2B** derivatives. Left) Bacteriorhodopsin crystal obtained with LC038, thanks to V. Gordeliy (IBS, France), (middle image) Cytochrome C crystals obtained with CALX004SFO, courtesy of A Jimi and P Crowley, University of Galway (right) ATP synthase crystal grown with LC001, thanks to M-F Giraud (IBGC, France)



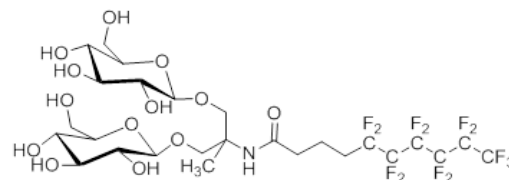
Chemical structures of The CALIXAR C2B Kit Additives



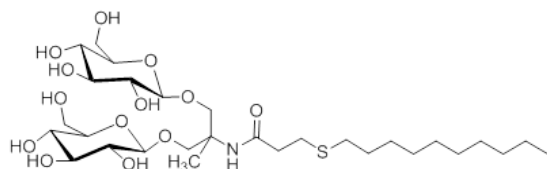
LC001



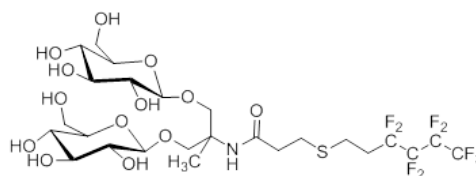
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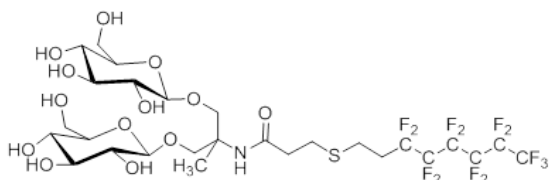
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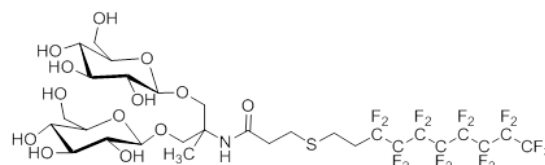
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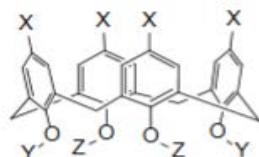
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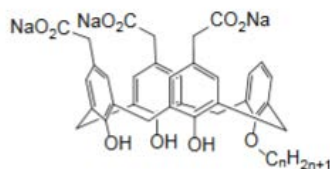
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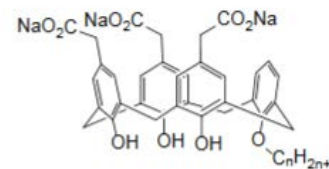
LC039



CALX004SFO

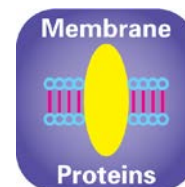


CALX113ACE



CALX163ACE

X -SO₃Na ; Y = Z = -H



The CALIXAR™ C2B Kit MD1-109

Table 1. CALIXAR C2B additives used in The CALIXAR™ C2B Kit.

The CALIXAR C2B Kit	mM (final concentrations)	MW (g/mol)	m(theo.) for 50 Kits (g)
LC001	10	2130	0.0533
LC002	10	2230	0.0558
LC021	10	817.5	0.0204
LC036	10	685.9	0.0171
LC037	10	763.6	0.0191
LC038	10	863.6	0.0216
LC039	10	963.7	0.0241
CALX004SFO	10	832.7	0.0208
CALX113ACE	10	678.6	0.0170
CALX163ACE	10	748.7	0.0187

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

Ordering Details:

Catalogue Description

The CALIXAR™ C2B Kit
The CALIXAR™ C2B Single Reagents (50 µL)

Catalogue Code

MD1-109
MDSR-109-tube number

Limited-Use Restriction (Important)

The purchase of **The CALIXAR™ C2B Kit** and its reagents product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact enquiries@moleculardimensions.com with the subject Out-licensing or write direct to Out Licensing, Molecular Dimensions Ltd, Unit 6, Goodwin Business Park, Willie Snaith Rd, Newmarket, Suffolk, CB8 7SQ.