

MemMagic™ Bicelle Screen kit

INSTRUCTION MANUAL

Catalog MX 201001 MemMagic™ Bicelle Screen kit (100 µl)
MX 201002 MemMagic™ Bicelle Screen kit (250 µl)

Revision A

For In Vitro Use Only



www.memxbio.com

ORDERING INFORMATION AND TECHNICAL SERVICES

P.O. Box 64-217
Los Angeles, CA 90064

Telephone (800) 817-7057
Order Toll Free (800) 817-7057
Technical Services (800) 817-7057
Fax (888) 912-9147

E-mail support@memxbio.com
World Wide Web www.memxbio.com

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MemMagic™ Bicelle Screen Kit

MATERIALS PROVIDED

Catalog # MX 201001

Materials provided (per kit)	Quantity
40% DMPC:CHAPSO (2.8:1)	100 µl
35% DMPC:CHAPSO (2.8:1)	100 µl
30% DMPC:CHAPSO (2.8:1)	100 µl
25% DMPC:CHAPSO (2.8:1)	100 µl

Catalog # MX 201002

Materials provided (per kit)	Quantity
40% DMPC:CHAPSO (2.8:1)	250 µl
35% DMPC:CHAPSO (2.8:1)	250 µl
30% DMPC:CHAPSO (2.8:1)	250 µl
25% DMPC:CHAPSO (2.8:1)	250 µl

STORAGE CONDITIONS

All Components: **Store at –20°C upon receipt.**

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INTRODUCTION

Crystallization remains an important bottleneck in membrane protein structure determination. While crystallization directly from detergents has been the traditional approach, the lipidic cubic phase method is becoming increasingly popular. However, both methods have significant limitations to be widely applicable to all membrane proteins. First of all, not all membrane proteins are stable and functional in detergents. Although the lipidic cubic phase method can improve membrane protein stability and functionality, it is tedious and laborious to setup crystallization experiments in the solid phase gel-like environment, therefore, making it incompatible with most currently available high-throughput robotic systems. This drawback has posed big challenges in screening broad crystallization conditions.

MemX Biosciences introduces MemMagic™ Bicelle Screen kit based on the use of bicelles as an alternative method for the crystallization of membrane proteins in a lipidic environment. Bicelles are disk-like micelles formed by the mixture of a phosphatidylcholine lipid such as Dimyristoyl-phosphatidylcholine (DMPC) and a detergent such as 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO). The bicelle discs can be described as patches of lipid bilayers with detergent molecules lining the apolar edges of each bilayer. Bicelles present a compromise between a rigid lipidic medium and an artificial detergent medium while offering beneficial aspects from both. Membrane proteins can be readily reconstituted into bicelles and are maintained in a native-like bilayer environment, which can be manipulated with almost the same ease as detergent solubilized membrane proteins, making it compatible with standard high-throughput screening. Recently, a significant number of membrane proteins have been successfully crystallized using the bicelle method, including Bacteriorhodopsin, β 2 Adrenergic receptor/Fab, Voltage-Dependent Anion Channel and Xanthorhodopsin.

MemMagic™ Bicelle Screen kit provides four bicelle solutions comprising of 40%, 35%, 30%, and 25% DMPC:CHAPSO (2.8:1). When mixed with protein in a ratio of 1:4 (bicelle:protein), these bicelle solutions enable the screening of 8%, 7%, 6% and 5% bicelle concentrations in the final protein-bicelle mixture, providing an optimal bicelle concentration range supporting successful crystal growth. MemMagic™ Bicelle Screen kit can be used with the standard commercially available crystallization screening kits. MemMagic™ Bicelle Screen kit can also be used in conjunction with nanoliter liquid handling robotic systems, such as the mosquito® by TTP Lab Tech.

PROTOCOL

1. When ready to use, thaw the bicelle solution at room temperature until it becomes a clear gel. Place the clear gel on crushed ice (not on cold blocks) to allow it to transform completely to liquid form. Vortex for 2-3 seconds and immediately return it to crushed ice to maintain its liquid form. MemMagic™ Bicelle solution is stable under multiple freeze-thaw processes. The bicelle solution should be vortexed after each freeze-thaw to re-establish a homogenous bicelle phase. The bicelle solution may become cloudy when placed on ice.

Note: MemMagic™ Bicelle solution has unique phase transition properties. It maintains liquid form at 4°C or on ice. It transforms to a clear gel at room temperature or higher. It solidifies when below 0°C.

2. Add the bicelle solution to the protein (preferably protein concentration >10 mg/ml) in a 1:4 (bicelle:protein) ratio (e.g. 10 µl bicelle + 40 µl protein) while keeping everything on ice.

Note: Only make enough protein/bicelle mixture for a single day experiment. **DO NOT** store protein/bicelle mixture for next day or future use.

Note: We recommend 1:4 (bicelle:protein) ratio to start. Other ratios can be performed as well, particularly in the optimization stage.

3. Mix by pipetting the contents up and down until the solution appears homogenous (**DO NOT VORTEX**).

4. Incubate the protein/bicelle mixture on ice for a minimum of 30 min before setting up crystallization trials in sitting drop or hanging drop format, using manual or standard high throughput robotic systems.

Note: **DO NOT** incubate crystallization trays at 4°C or below. Bicelles in the crystallization drops may precipitate under such temperature conditions.

Note: Some conditions in many commercially available crystallization screens are not fully compatible with bicelle solutions, resulting in a high incidence of false positives (lipid crystals or salt crystals). We recommend using additional tools, such as a UV microscope, to rule out such false positives before proceeding to optimization step.

REFERENCES

Faham, S., Ujwal, R., Abramson, J. and Bowie, J. U. (2009) “Practical Aspects of Membrane Proteins Crystallization in Bicelles” Current Topics in Membranes, Volume 63, Chapter 5, 111-127.

Faham, S., Boulting, G. L., Massey, E. A., Yohannan, S., Yang, D., & Bowie, J. U. (2005). “Crystallization of bacteriorhodopsin from bicelle formulations at room temperature”. Protein Science, 14, 836–840.

Faham, S., & Bowie, J. U. (2002). “Bicelle crystallization: A new method for crystallizing membrane proteins yields a monomeric bacteriorhodopsin structure”. Journal of Molecular Biology, 316, 1–6.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information is provided on MemX Biosciences website at <http://www.memxbio.com>. MSDS documents are not included with product shipments.