BMA101 (DIBMA) User Guide

Despite the wide use of conventional detergents, removal of a membrane protein from the native lipid bilayer and into a detergent micelle (Figure 1) can possibly interfere with the structure, function, and stability of the protein [1,2]. To avoid some of these issues with detergents, many systems have been developed to mimic the native lipid membrane. These include bicelles (Figure 2)[3], MSP-nanodiscs (Figure 3)[4], and detergent-free polymer systems such as styrene-maleic acid co-polymers (SMAs)[5] and diisobutylene-maleic acid (DIBMA)[6,7] (Figure 4). DIBMA from Anatrace is a highly purified version of diisobutylene and maleic acid co-polymer in a 1:1 ratio.

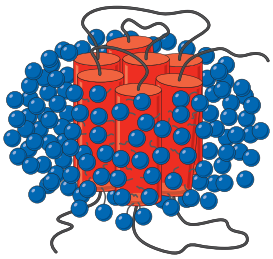

Figure 1

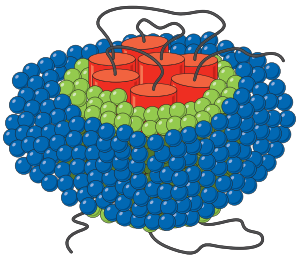

Figure 2

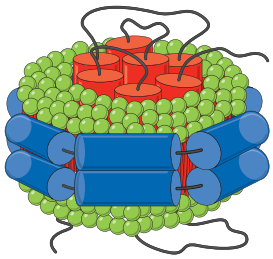

Figure 3

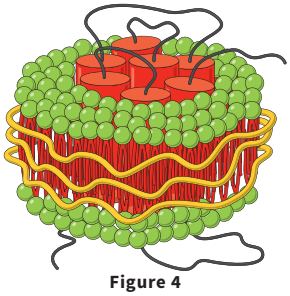

Figure 4

This user guide contains a general protocol and suggestions on how to use the DIBMA polymer to solubilize, purify, and characterize membrane proteins.

Product Information:

- SKU: BMA101
- Name: Diisobutylene Maleic Acid co-polymer, sodium salt / DIBMA / Purified Sokalan® CP9[8]
- MW: ~12,000 g/mol
- dn/dc: 1.346 M^{-1}
- Solubility: > 10% in H_2O
- Absorbance at 280 nm (1% solution): < 0.3
- Mg^{2+} Tolerance: 25 mM
- Ca^{2+} Tolerance: 20 mM

DIBMA Protocol:

- To solubilize membrane proteins from the native lipid bilayer, DIBMA can be added directly to a preparation of isolated membranes that have been resuspended in buffer. The DIBMA powder can be added to the membranes to a final concentration of 1% – 3%.
- Alternatively, a stock solution of DIBMA in buffer can be prepared and added to resuspended membranes to a final concentration of 1% - 3%.
- Solubilization can be performed at room temperature or at 4°C with gentle rocking. When compared to solubilization with other polymers, such as SMA, typically a slightly longer solubilization time is needed for optimal extraction efficiency.

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BMA101 (DIBMA) User Guide

- The pH of the solubilization buffer has been shown to effect solubilization efficiency[6], thus small scale solubilization experiments should be performed to determine the optimal pH.
- Upon solubilization, no additional DIBMA polymer needs to be present in buffers used for protein purification, and biophysical analysis.
- Solubilized membrane proteins in DIBMA are compatible with all commonly used protein purification techniques, including IMAC, affinity, ion exchange, and SEC.
- Membrane proteins solubilized and purified in DIBMA are compatible with most biophysical characterization assays, including fSEC, SEC-MALS, UV/Vis spectroscopy, western blotting, and SDS-PAGE (see below).
- Compared to SMA, the absorbance of DIBMA at 280 nm is significantly lower due to the absence of aromatic groups. Additionally, DIBMA is tolerant to higher concentrations of divalent cations.
- The addition of DIBMA to solubilize membrane proteins from whole cells has been performed with limited success.

SDS-PAGE Protocol:

The presence of polymers (both SMA and DIBMA), may result in the smearing of bands on SDS-PAGE gels[6, 9]. Proteins can be separated from polymers through a simple precipitation using methanol, chloroform, and water. This protocol was originally described in[10], and slightly modified in[6] for use with the DIBMA polymer.

- Measure volume of polymer-containing protein sample
- Add 4x volume of cold methanol to sample and mix thoroughly via vortexing
- Add 1x volume of cold chloroform to sample and mix thoroughly via vortexing
- Add 3x volume of cold water, mix, and centrifuge for 2 min at 4°C and 14,000 g.
- Remove top aqueous layer, add 4x volume of methanol, and mix
- Pellet proteins by centrifuging at 5,000 g for 1 min and 20,000 g for 5 min at 4°C
- Remove supernatant and dry pellet
- Add SDS loading buffer, run SDS-PAGE as normal.

References:

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