anatrace
A CALIBRE SCIENTIFIC COMPANY

User Guide

SMA-EA, SMA-ED, SMAd-A, SMA-QA

NEW STYRENE-MALEIC ANHYDRIDE (SMA) POLYMERS

Detergent-free system for structural and functional studies of membrane proteins

NON-DENATURING | NANODISC OF NATURAL OR SYNTHETIC LIPIDS | ADJUSTABLE NANODISC SIZE | STABLE AT HIGH CA^{2+}/MG^{2+}

Anatrace offers next-generation polymer derivatives of styrene maleic acid (SMA), which show increased stability towards higher concentrations of divalent cations (up to 200 mM Ca^{2+} or Mg^{2+}) and allow for various sizes of lipid nanodiscs to be utilized by changing the lipid-to-polymer ratio. The resulting polymers have been shown to form nanodiscs with synthetic lipids [1] and directly solubilize membrane proteins from membranes [2]. These new polymers are a great alternative to detergents, which often interfere with the structure and function of proteins and may cause denaturing. The solubilization protocol is easy and straightforward (see Fig. 1).

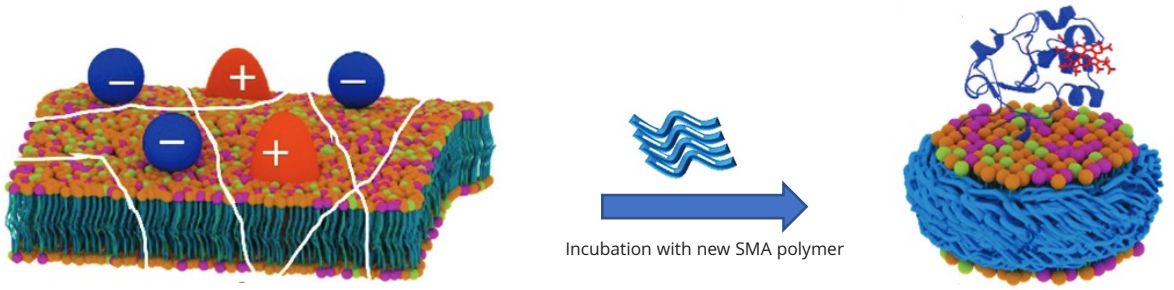

Figure 1. Direct extraction of a membrane protein in native cell membrane lipids. Adapted from [2].

Storage Conditions

2-8°C for long-term storage.

Direct Membrane Protein Solubilization Protocol

- To solubilize membrane proteins from the native lipid bilayer, the polymer can be added directly to a preparation of isolated membranes that have been resuspended in buffer to a final concentration of 1% – 3%. The polymer can be added as a powder or a stock solution of SMA in a buffer (see Fig. 2 for pH stability of new SMA polymers; the optimal pH in each case may be slightly different).
- Solubilization is performed at room temperature or at 4°C while gently rocking the solution.
- Membrane proteins solubilized in SMA polymers are compatible with all commonly used protein purification techniques, including IMAC, affinity, ion exchange, and SEC.
- Membrane proteins solubilized and purified in SMA are compatible with most biophysical characterization assays, including SEC, SDS-PAGE, DLS, TEM, and NMR spectroscopy.

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Reconstitution of Membrane Protein into Prepared DMPC Nanodiscs

- Nanodiscs with different polymers are prepared using DMPC (10 mg/ml) in 50 mM potassium phosphate at pH 7.4 as stock solution.
- Polymer stock solutions are prepared as 10 mg/ml in buffer (for pH choice, see Fig. 2).
- SMA-EA nanodiscs are prepared by the addition of 2:1 w/w SMA-EA (10 mg/ml) and DMPC (10 mg/ml), followed by incubation at 37˚C for 4 hrs. SMA-QA nanodiscs are prepared by adding 1.5:1 w/w SMA-QA (10 mg/ml) and DMPC (10 mg/ml), followed by incubation at 37˚C for 4 hrs.
- Nanodiscs can be subsequently purified using size-exclusion chromatography and their size can be assessed using dynamic light scattering.
- We recommend incubating the target protein for 12 h with nanodisc solution (100:1 DMPC:protein) in 50mM potassium phosphate buffer (pH 7.4).

Ordering Information

Item #	Description	UOFM	UOM 2021
SMA-EA 1 G	SMA-EA	EA	$365
SMA-EA 500 MG	SMA-EA	EA	$205
SMA-EA 250 MG	SMA-EA	EA	$125
SMA-ED 1 G	SMA-ED	EA	$560
SMA-ED 500 MG	SMA-ED	EA	$310
SMA-ED 250 MG	SMA-ED	EA	$175
SMAd-A 1 G	SMAd-A	EA	$850
SMAd-A 500 MG	SMAd-A	EA	$475
SMAd-A 250 MG	SMAd-A	EA	$265
SMA-QA 1 G	SMA-QA	EA	$2,375
SMA-QA 500 MG	SMA-QA	EA	$1,310
SMA-QA 250 MG	SMA-QA	EA	$725

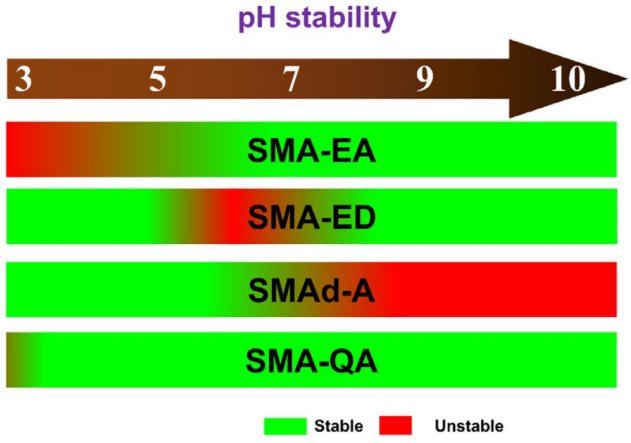

Figure 2. pH stability chart for new SMA polymers [3].

References

1. Ravula, T. et al. Effect of polymer charge on functional reconstitution of membrane proteins in polymer nanodiscs. Chem Commun 54, 9615–9618 (2018).
2. Krishnarjuna, B. et al. Detergent-free extraction, reconstitution and characterization of membrane-anchored cytochrome-b5 in native lipids. Chem Commun 56, 6511–6514 (2020).
3. Ravula, T., et al. Styrene maleic acid derivates to enhance the applications of bio-inspired polymer based lipid-nanodiscs. Eur Polym J 108, 597–602 (2018).

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