

## SEPAX TECHNOLOGIES TweenTrap Column

## Experiencing issues of surfactants interfering or coeluting with your targeted biomolecules on your analytical size exclusion runs?

The Sepax TweenTrap column is specifically designed for trapping surfactants, such as Polysorbate 80/20 (PS 80/20, Tween 80/20), in aqueous samples. The packing support is composed of rigid, spherical, highly cross-linked poly(styrene divinylbenzene) (PS/DVB) beads with a particle size of 5  $\mu$ m and a proprietary surface coating is covalently bonded onto the PS/DVB support. TweenTrap columns can be run in tandem with SEC columns in aqueous samples, and are specifically designed to trap and remove surfactants, such as Polysorbate 80/20, which can interfere with quantitative protein SEC analysis.

2.5

## **COLUMN RECOMMENDATIONS**

- Run the TweenTrap column with SEC column in aqueous based mobile phases, without the addition of organic solvents.
- Perform CIP wash every 20 injections, when the concentration of tween is 0.01% in sample; disconnect the TweenTrap column from the SEC column before the cleaning
- Trapped surfactants can be cleaned with one of two methods:
  - Organic solvents, such as IPA: reverse flow direction at 0.35 mL/ min for 15 minutes followed by 10 min wash with water
  - · A caustic 0.5 M NaOH: reverse flow direction at 0.35 mL/min for 15 minutes followed by a 30 min wash with water



Sepax Part #: 010054-4603 Particle Size: 5 μm Pore Size: Non-Porous ID x Length: 4.6 x 35 mm

Detailed guidelines can be found in the TweenTrap User Manual

The chromatographic overlay shown below, in **Figure 1**, helps to illustrate how Tween 80 in the formulation of Erbitux can be trapped onto the Sepax TweenTrap column, while still allowing for effective aggregate peak detection and integration.

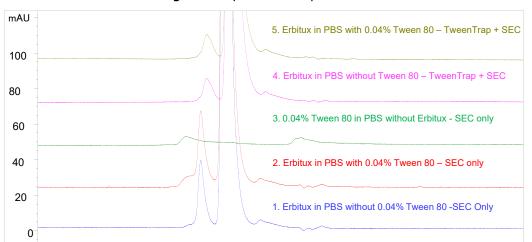


Figure 1. Sepax TweenTrap Column

Zenix-C SEC-300 Column: 7.8x 300 mm SS, TweenTrap Column: 4.6 x 35 mm SS; Mobile phase: 150 mM Sodium Phosphate Buffer pH 7.0; Detector: 214 nm; Flow rate: 1 mL/min; Column temperature: ambient; Injection: 100  $\mu$ L

**Trace 1** shows the SEC analysis of Erbitux alone without Tween 80. Clear aggregate peaks are seen in front of the monomer.

**Trace 2** shows the SEC analysis of Erbitux in 0.04% Tween 80, without a TweenTrap column. Attention should be drawn to the front shoulder of the aggregate region, which can be attributed to Tween 80, based on the profile of an injection of Tween 80 alone (**Trace 3**). Indicating that there is possible Tween 80 interference in the quantitation of Erbitux (**Trace 2**).

With the addition of a Sepax TweenTrap column, **Trace 5** illustrates a profile where Tween 80 interference is minimized. This is further confirmed by an injection just Erbitux, with no Tween 80 formulation, onto the same column setup (**Trace 4**).

As always, our technical support team is here to help! 1-877-SEPAX-US | www.sepax-tech.com | @SepaxTech