

## PEGylated L-Chymotrypsinogen A (N-Terminal PEG 20KDa) on BioSep2000 (2)

**Column:** BioSep™ 5 µm SEC-s2000 145 Å, LC Column 300 x 7.8 mm, Ea

**Dimensions:** 300 x 7.8 mm ID

**Order No:** 00H-2145-K0

**Elution Type:** Isocratic

**Eluent A:** 100mM Phosphate buffer pH 6.8□□

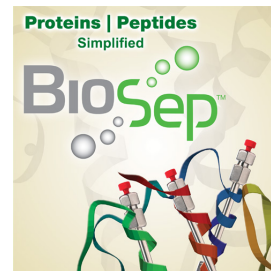
Gradient	Step No.	Time (min)	Pct A
Profile:	1	0	100

**Flow Rate:** 1 mL/min

**Col. Temp.:** ambient

**Detection:** UV-Vis Abs.-Variable Wave.(UV) @ 220 nm (ambient)

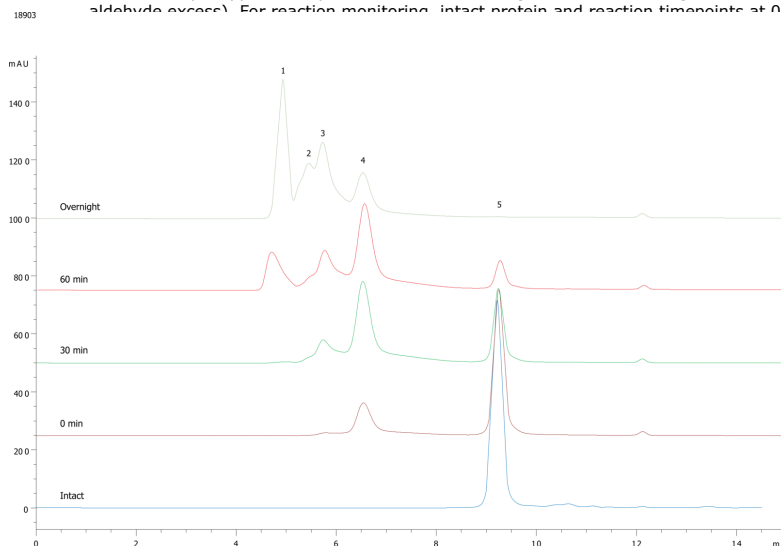
**Analyst Note:** Application Topic: Monitoring protein PEGylation and purifying PEGylated proteins from their reaction



Products used in this application:



Therapeutic proteins are often PEGylated to increase their serum lifetime; however, such reactions typically generate a heterogeneous product that can be difficult to characterize and purify. PEGylated proteins are usually purified by GFC or RPC after synthesis and the PEGylation reaction is monitored by HPLC. In this example application, protein was reacted using N-terminal favoring conditions (phosphate pH 6.5 with cyanoborohydrate and 5% PEG 20KDa aldehyde excess). For reaction monitoring, intact protein and reaction timepoints at 0 minutes, 30 minutes, 60 minutes and an overnight reaction



### ANALYTES:

- 1 4 PEG / Chymo A complex
- 2 3 PEG / Chymo A complex
- 3 2 PEG / Chymo A complex
- 4 PEGylated Chymotrypsinogen A
- 5 Chymotrypsinogen A

