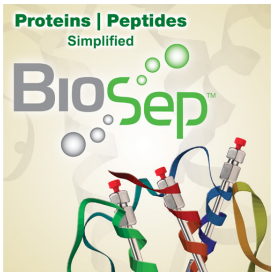
HPLC Application

ID No.: **18903**

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

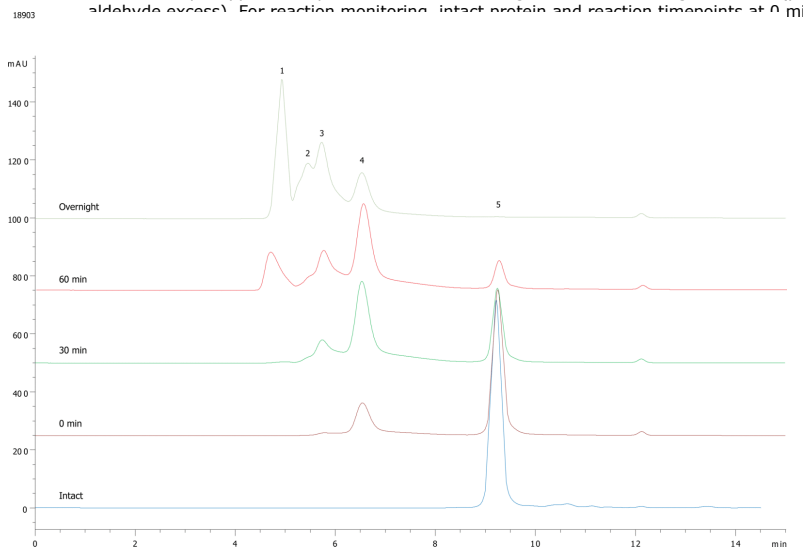


Products used in this application:

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

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 In this example application, protein was reacted using N-terminal favoring conditions (phosphate pH 6.5 with cyanoborohydrate and 5x PEG 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

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