protein order 123546

Organization	**ATUM**
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Start date	**11/07/2018**
Confirmed date	**11/08/2018**

sample summary

Protein ID	Protein Name	Conc. (mg/ml)	Yield (mg)	Culture Volume (ml)	Endotoxin (EU/mg)	Titer (mg/L)	Aliquots	Volume (ml)
12345.1.a	Cytokine	1.63	68.46	2000	< 0.34	34.23	4	10.00
							1	2.00

12345.1.a

Protein Name	**Cytokine**
Molecular Weight	**49020 Da**
Extinction Coefficient	**76945**
Isoelectric Point	**8.60**
Date of Manufacture	**11/10/2018**

production notes

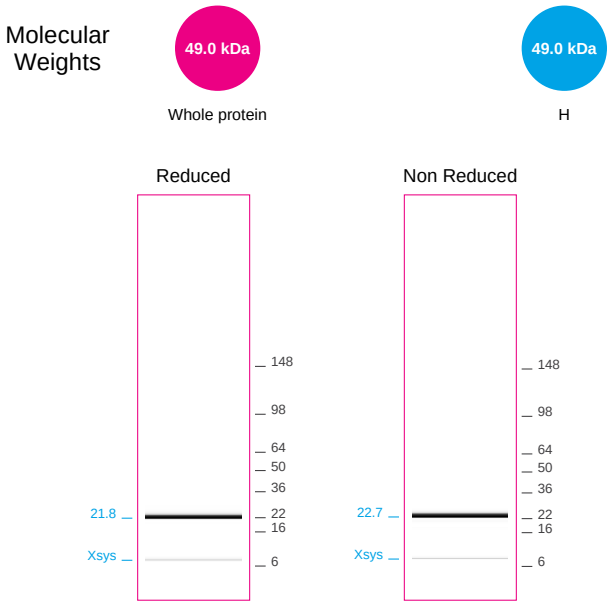
Expression Host:	
Culture Volume:	**2000 ml**
Purification resins:	
Endotoxin:	**< 0.34**
Yield:	**68.46 mg**
Formulation Buffer:	**20 mM Histidine, 150 mM NaCl, pH 6.0**

productivity data

Titer: **34.23 mg/L**

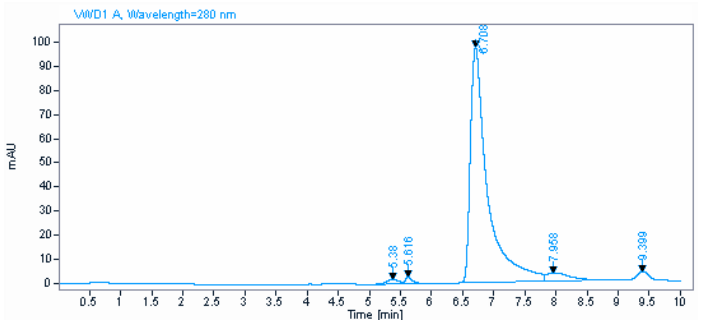
identity

SDS-PAGE - Product Identity



biophysical data

SEC - Product Characterization



Retention time (min)	Area %	Peak symmetry
5.38	1.35	1.04
5.62	1.19	0.37
6.71	90.47	0.38
7.96	4.69	0.32
9.40	2.30	0.88

ATUM production report

1. Protein expression vectors were transformed into E.coli using standard ATUM methods

2. From a single colony a 2 x 1 liter Expression cutlure was setup and induced by Autoinduction.

3. After expression the cells were lyzed by sonication

4. His60 resin was used to capture proteins

5. Resin was washed 20mM Hepes, 500mM NaCl, 20mM Imidazole

6. Proteins were eluted with 20mM Hepes, 500mM NaCl, 250mM Imidazole

7. Proteins were buffer exchanged in to 20 mM Histidine pH 6.0, 150 mM NaCl

8. Endotoxin was then removed from the sample

9. Protein was quantified by OD280, quantity and concentration was determined using calculated extinction coefficient.

10. Reduced and non-reduced SDS-PAGE (Biorad criterion Tris/Glycine/SDS, 4-20%) or Perkin Elmer GXII capillary electrophoresis system, was used to determine purity and approximate molecular mass.

11. Pierce Endotoxin Quantitation Kit or Charles River Nextgen PTS was used to determine endotoxin levels.

12. Aggregation status was determined by HPLC, with detection at 280nm using a Sepax Zenix-C SEC-300, 3um, 300Å, 4.6*150mm size exclusion column and PBS running buffer

13. Proteins were shipped as aliquots after filter sterilization, snap frozen in liquid nitrogen.

protein information

Molecular Weight and Extinction Coefficient are estimated for the sum of the contributing protein chains in the quaternary structure. By default the calculation assumes equal and monomeric contribution from each chain.

Extinction Coefficient is the predicted absorbance at 280nm per molar protein in units of $M^{-1}cm^{-1}$.

Potential post-translational modifications such as glycosylation, phosphorylation, and proteolysis are not considered in Molecular Weight or Extinction Coefficient estimates.

protein analytics

SDS-PAGE gels are run under reducing and non-reducing conditions to determine purity and approximate molecular mass. Digital data results are collected, reviewed and exported into our LIMS system. The data is then displayed and exported as virtual gels.

Size Exclusion Chromatography (SEC) – HPLC separates molecules based on molecular mass and hydrodynamic volume. Larger molecules elute first i.e. earlier retention time, followed by smaller sized molecules i.e. later retention time. The table shows retention time, area % and peak symmetry. Area % of each peak is calculated based on total area of the peaks. Peak symmetry is calculated to determine the peak fronting (<1) or tailing (>1).