Figure 1.

- **Anti-IL-13 Antibody Heavy Chain Variable Region Sequence**

```
1         10         20         30         40
EVTLRESGPG LVKPTQTLTL TCTLYGFSLS TSDMGVDWIR
                                    CDR1

QPPGKGLEWL AHIWWDDVKR YNPALKSRLT ISKDTSKNQV
                    CDR2

VLKLTSVDPV DTATYYCART VSSGYIYYAM DYWGQGTLVT
                          CDR3

VSS
```

- **Anti-IL-13 Antibody Light Chain Variable Region Sequence**

```
1         10         20         30         40
DIQMTQSPSS LSASVGDRVT ISCRASQDIR NYLNWYQQKP
                             CDR1

GKAPKLLIFY TSKLHSGVPS RFSGSGSGTD YTLTISSLQP
              CDR2

EDIATYYCQQ GNTLPLTFGG GTKVEIK
              CDR3
```

Figure 2. Cell Culture Process Flow Diagram

Cell Culture Step	Set point/target	In-Process Controls Test	Action Limit
Master Cell Bank	Storage temp ≤ -130°C	Viability at thaw	≥ 80%
↓			
1 x 125 mL Disposable Spinner Flask	Temperature 37°C 5% CO_2	Culture viability at transfer	≥ 80%
↓			
1 x 125 mL Disposable Spinner Flask	Temperature 37°C 5% CO_2	Culture viability at transfer	≥ 80%
↓			
3 x 125 mL Disposable Spinner Flask	Temperature 37°C 5% CO_2	Culture viability at transfer	≥ 80%
↓			
1 x 2 L Biowave Bag	Temperature 37°C 5% CO_2	Culture viability at transfer	≥ 80%
↓			
1 x 20 L Biowave Bag	Temperature 37°C 5% CO_2	Culture viability at transfer	≥ 80%
↓			
2 × 20 L Biowave Bags	Temperature 37°C 5% CO_2 Culture viability at transfer	Culture viability at transfer Viable cell count at transfer Contamination check	≥ 80% ≥ 2.0×10^6/mL No growth
↓			

Figure 2. Cell Culture Process Flow Diagram (Continued)

Cell Culture Step	Set point/target	In-Process Controls Test	Action Limit
110 L Seed Bioreactor	Temperature 37°C pH 7.1 40% DO	Viable cell count at transfer Contamination check	$\geq 2.6 \times 10^6$/mL No growth
↓			
3000 L Production Bioreactor (Short fill)	Temperature 37°C pH 7.1 40% DO	Viable cell count at end of short fill	$\geq 1.6 \times 10^6$/mL
↓			
3000 L Production Bioreactor	Temperature 35 → 33°C pH 6.9 40% DO	Viable cell count at temperature shift *Daily Samples:* Daily cell count Glucose concentration Poros A HPLC (ABT-308 titer) *Harvest sample (unprocessed bulk):* Harvest viability Poros A HPLC (ABT-308 titer) Endotoxin TEM Mycoplasma Adventitious viruses Q-PCR for MVM Contamination check	$\geq 3.0 \times 10^6$/mL Report value Report value Report value ≤ 50% Report value ≤ 5 EU/mL $\leq 10^8$ virus-like particles/mL Negative[a] No evidence of viral contamination[a] Negative[a] No growth[a]

Figure 3. Comparision of Alternative Cell Culture Process Flow Strategies

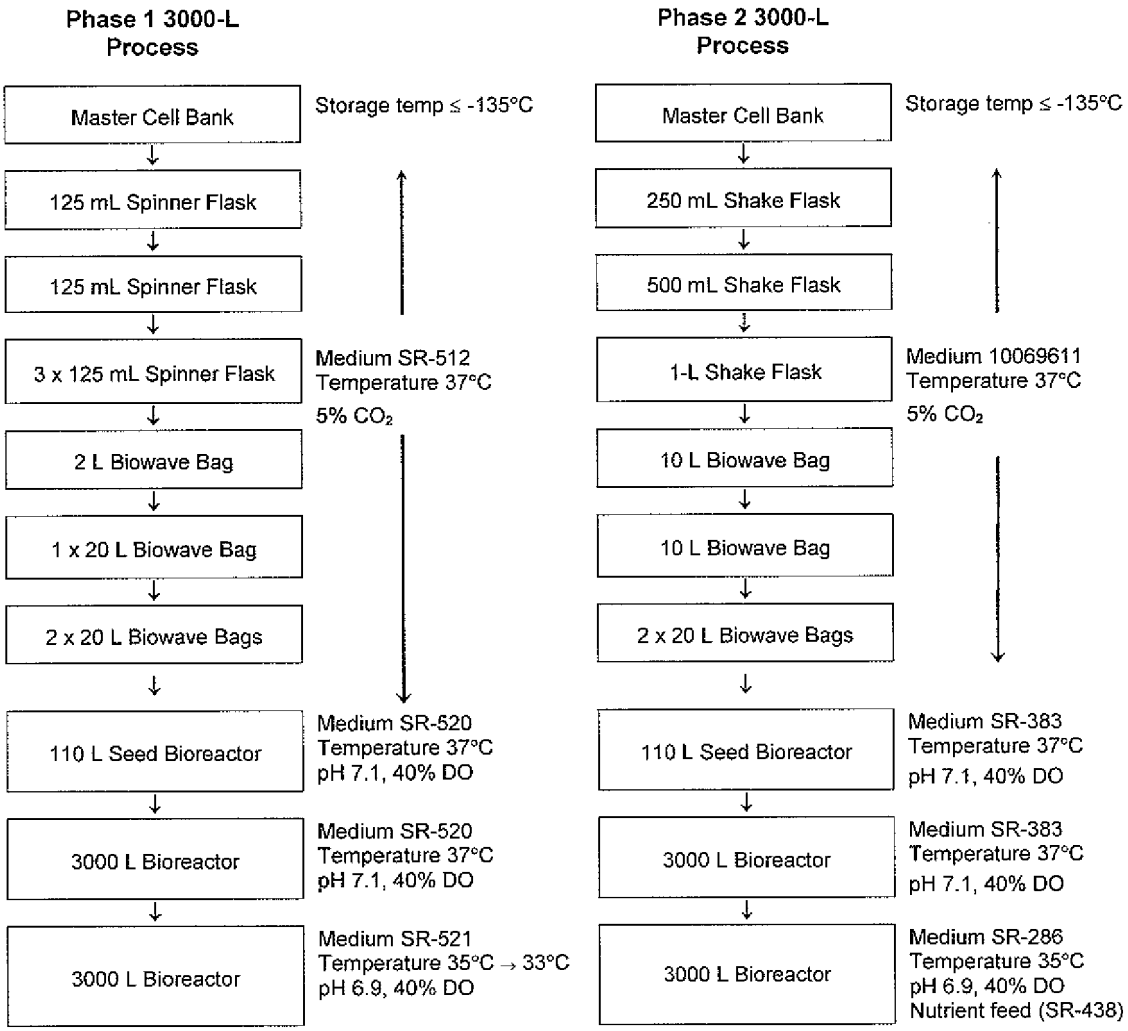


Figure 4. Primary Recovery and Capture Chromatography Process Flow Diagram

Step	Set point/target	In-Process Controls Test	Action Limit
Primary Recovery and Harvest Clarification	Ambient temperature	Poros A HPLC	Recorded to calculate mass
	10-25 L/min	Bioburden[a]	≤ 15.0 CFU/mL
	Depth and membrane filters	Endotoxin[a]	≤ 5 EU/mL
Protein A Affinity Chromatography	Ambient temperature	A_{280}	Recorded to calculate mass
	45 × 22 cm column (35 L)	SE-HPLC	≥ 90.0% Monomer
	Flow rate 150 – 300 cm/hr	Bioburden[a]	≤ 15.0 CFU/mL
	Elution buffer pH 3.5	Endotoxin[a]	≤ 5 EU/mL
	Load ≤ 32 g protein/L resin		
	Hold eluate at ambient		
Low pH Treatment and Filtration	Ambient temperature	A_{280}	Recorded to calculate mass
	pH 3.5, 60 - 70 min	SE-HPLC	≥ 90.0% Monomer
	Adjust to pH 5	Bioburden[a]	≤ 15.0 CFU/mL
	Depth and membrane filters	Endotoxin[a]	≤ 5 EU/mL

a. Sample taken at the start of the next unit operation.

Figure 5. Comparision of Alternative Primary Recovery and Capture Flow Strategies

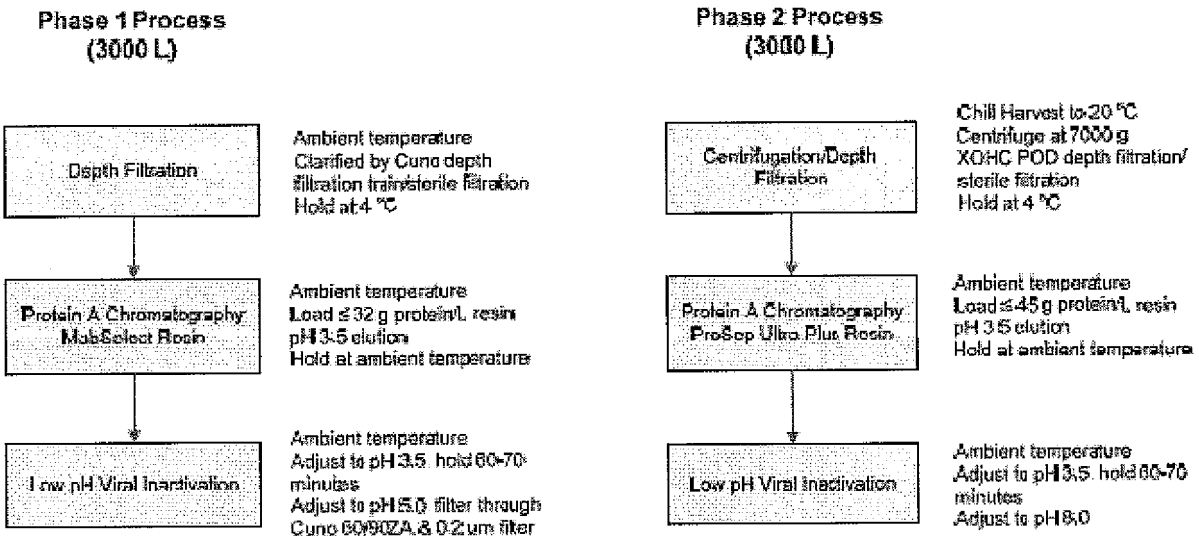
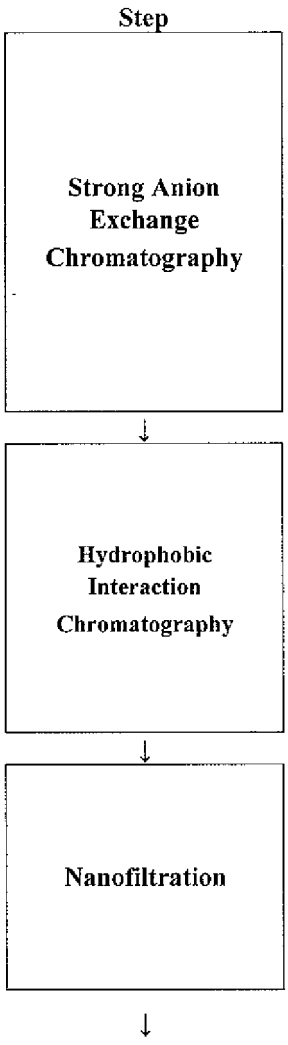


Figure 6. Fine Purification Process Flow Diagram



Step	Set point/target	In-Process Controls Test	Action Limit
Strong Anion Exchange Chromatography	Temperature 10-14°C	Load A_{280}	Recorded to calculate mass
	Adjust to pH 8	SE-HPLC	≥ 90.0% Monomer
	Adjust conductivity to 5.0 – 6.5 mS/cm	Bioburden[a]	≤ 15.0 CFU/mL
	Delipid and membrane filters	Endotoxin[a]	≤ 5 EU/mL
	45 × 22 cm column (35 L)		
	Flow rate 100 cm/hr		
	Load ≤ 80 g protein/L resin		
Hydrophobic Interaction Chromatography	Temperature 10-14°C	Load A_{280}	Recorded to calculate mass
	60 × 15 cm column (42 L)	Eluate A_{280}	Recorded to calculate mass
	pH 7	SE-HPLC	≥ 90.0% Monomer
	Flow rate 20 - 75 cm/hr	Bioburden[a]	≤ 15.0 CFU/mL
	Load ≤ 64 g protein/L resin	Endotoxin[a]	≤ 5 EU/mL
Nanofiltration	Temperature 10-14°C	Filtrate A_{280}	Recorded to calculate mass
	Pressure ≤ 34 psig	SE-HPLC	≥ 90.0% monomer
		Bioburden[a]	≤ 15.0 CFU/mL
		Endotoxin[a]	≤ 5 EU/mL
		Filter Integrity	≥ 32.8 mL/min @ 85 psig (UDV20)
			≥ 17.0 mL/min @ 85 psig (DV20)

a. Sample taken at the start of the next unit operation.

Figure 6. Fine Purification Process Flow Diagram (Continued)

Step	Set point/target	In-Process Controls Test	Action Limit
UF/DF	Temperature 10-14°C	A_{280}	Recorded to calculate mass
	Diafilter into 15 mM Histidine, pH 5.6	SE-HPLC	≥ 90.0% monomer
	Target concentration 120 - 160 g/L	Bioburden[a]	≤ 15.0 CFU/mL
		Endotoxin[a]	≤ 5 EU/mL
↓			
Filter, Bottle And Freeze Drug Substance	Temperature 2-8°C	A_{280}	Recorded to calculate mass
	Freeze -80°C (nominal)	Bioburden	≤ 1 CFU/mL[c]
	Concentration 120-160 g/L	Endotoxin[b]	≤ 0.2 EU/mg[c]
		Filter Integrity[b]	Bubble point ≥ 50 psid

a. Sample taken at the start of the next unit operation.
b. Drug substance test results.
c. Drug substance specifications

Figure 7. Comparision of Alternative Fine Purification Flow Strategies

