Figure 1

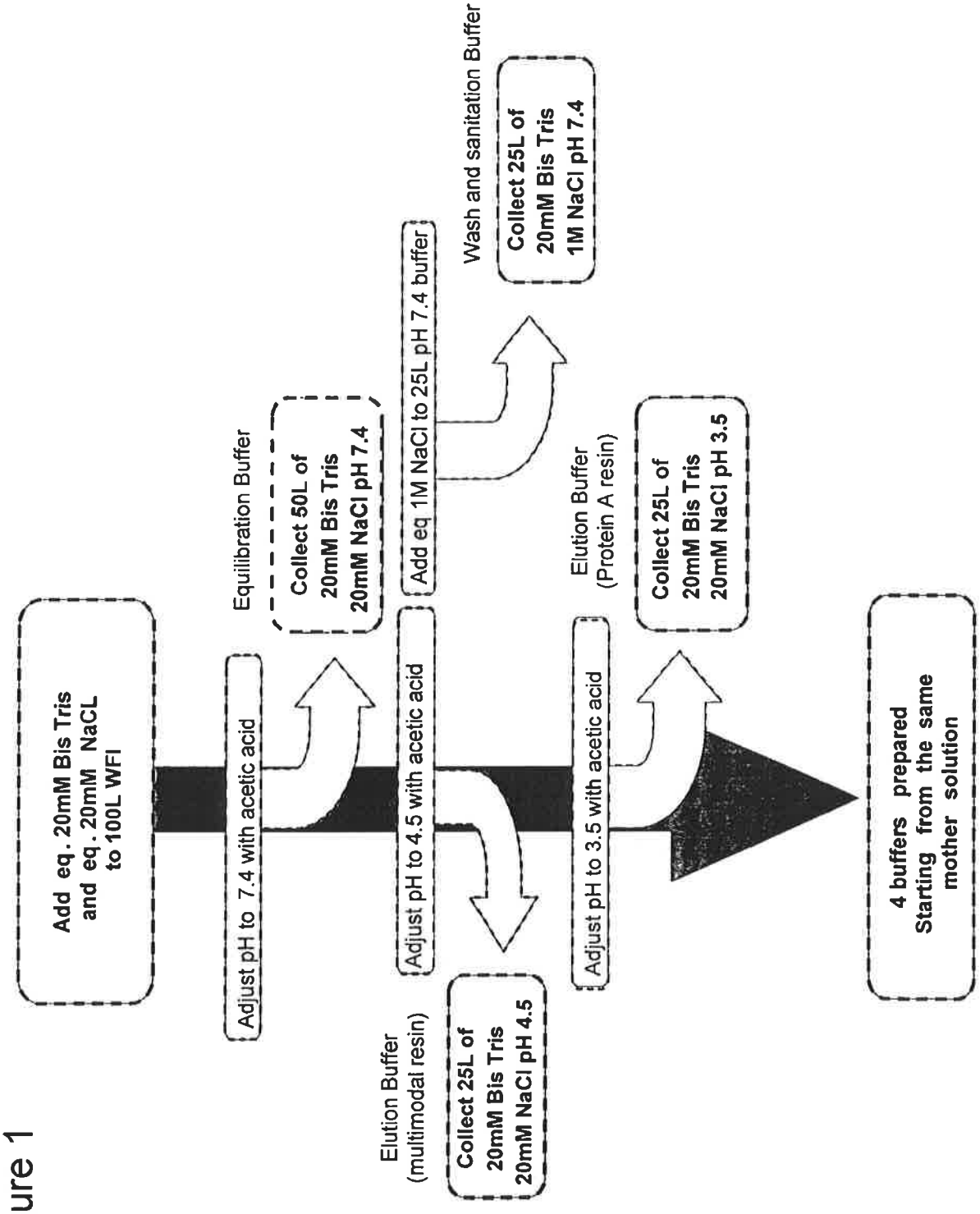

Add eq. 20mM Bis Tris and eq. 20mM NaCL to 100L WFI
Adjust pH to 7.4 with acetic acid
Equilibration Buffer
Collect 50L of 20mM Bis Tris 20mM NaCl pH 7.4
Adjust pH to 4.5 with acetic acid
Elution Buffer (multimodal resin)
Collect 25L of 20mM Bis Tris 20mM NaCl pH 4.5
Add eq 1M NaCl to 25L pH 7.4 buffer
Wash and sanitation Buffer
Collect 25L of 20mM Bis Tris 1M NaCl pH 7.4
Adjust pH to 3.5 with acetic acid
Elution Buffer (Protein A resin)
Collect 25L of 20mM Bis Tris 20mM NaCl pH 3.5
4 buffers prepared Starting from the same mother solution

Figure 2

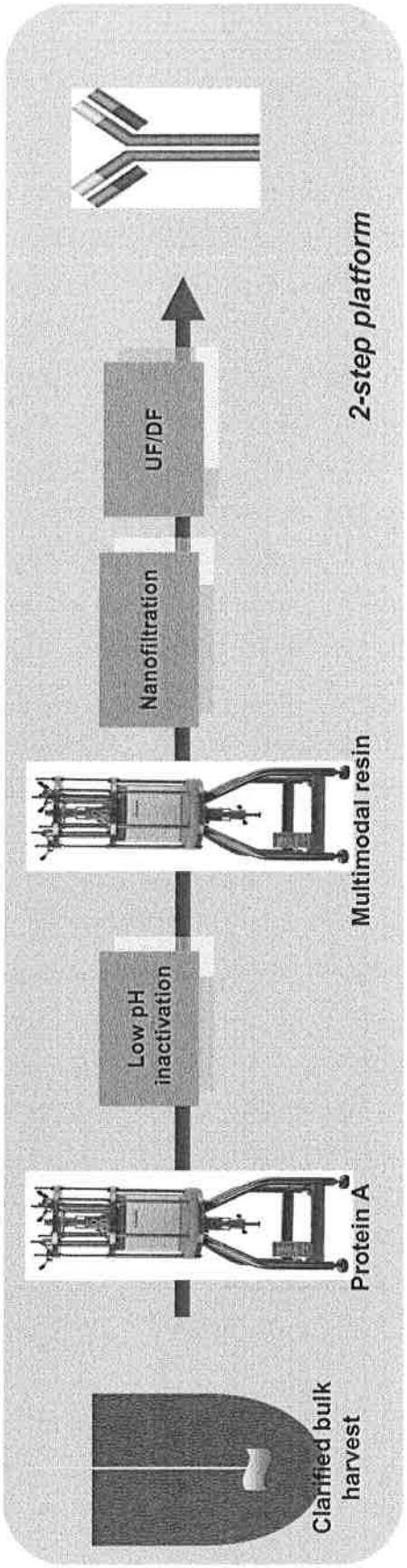

Clarified bulk harvest
Protein A
Low pH inactivation
Multimodal resin
Nanofiltration
UF/DF
2-step platform

Figure 3

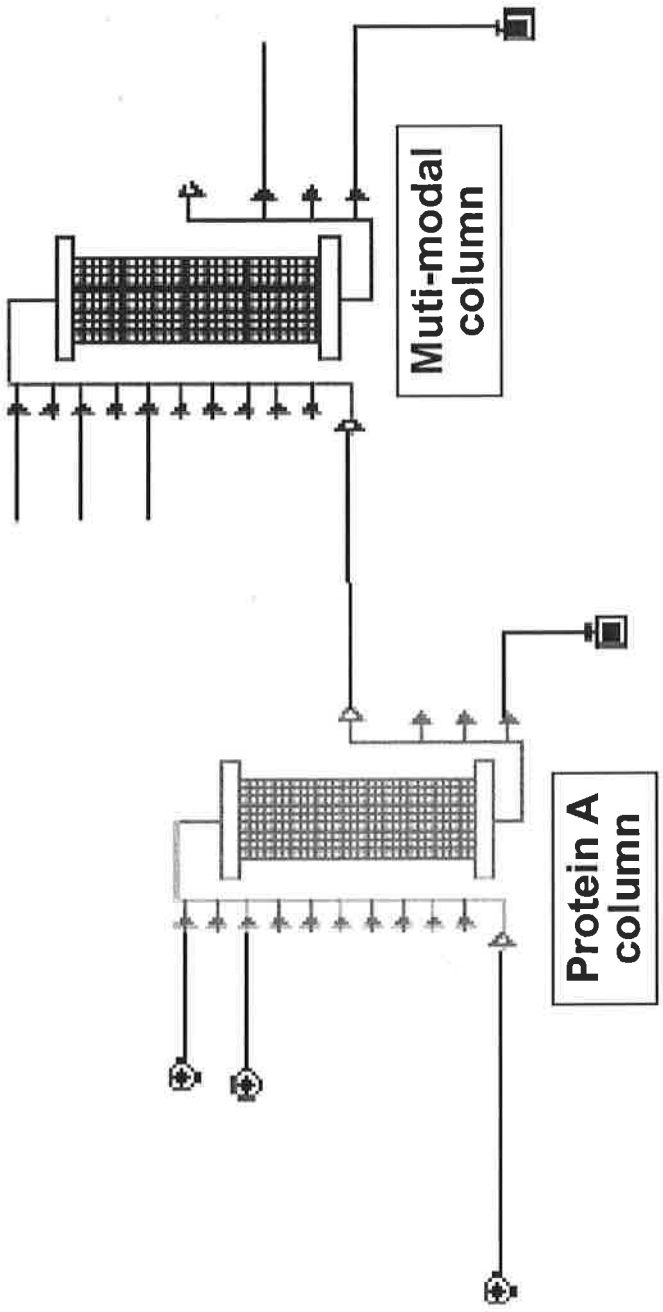


Figure 4

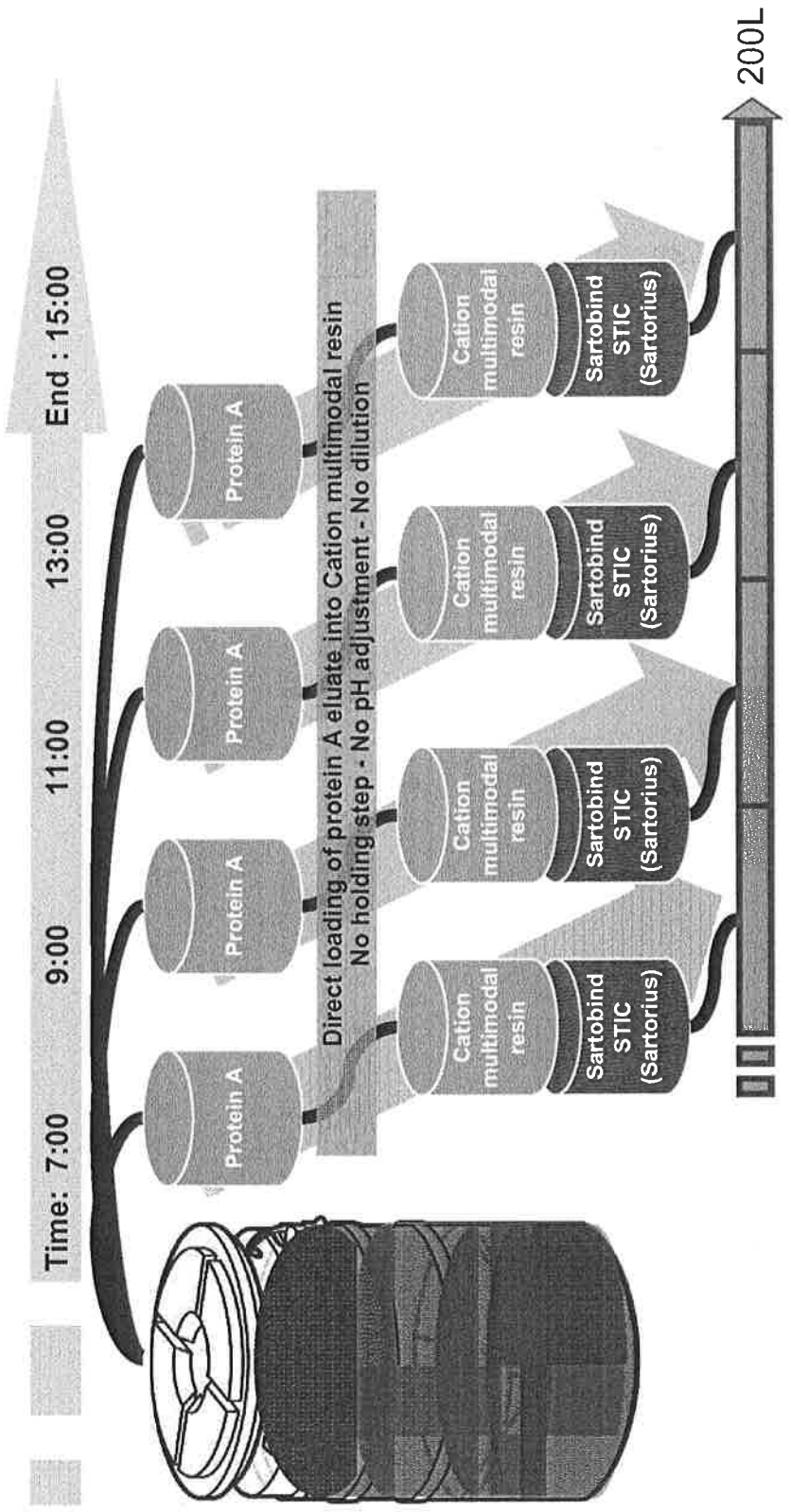

Time: 7:00
9:00
11:00
13:00
End : 15:00
200L
Protein A
Protein A
Protein A
Protein A
Direct loading of protein A eluate into Cation multimodal resin
No holding step - No pH adjustment - No dilution
Cation multimodal resin
Sartobind STIC (Sartorius)
Cation multimodal resin
Sartobind STIC (Sartorius)
Cation multimodal resin
Sartobind STIC (Sartorius)
Cation multimodal resin
Sartobind STIC (Sartorius)

Figure 5

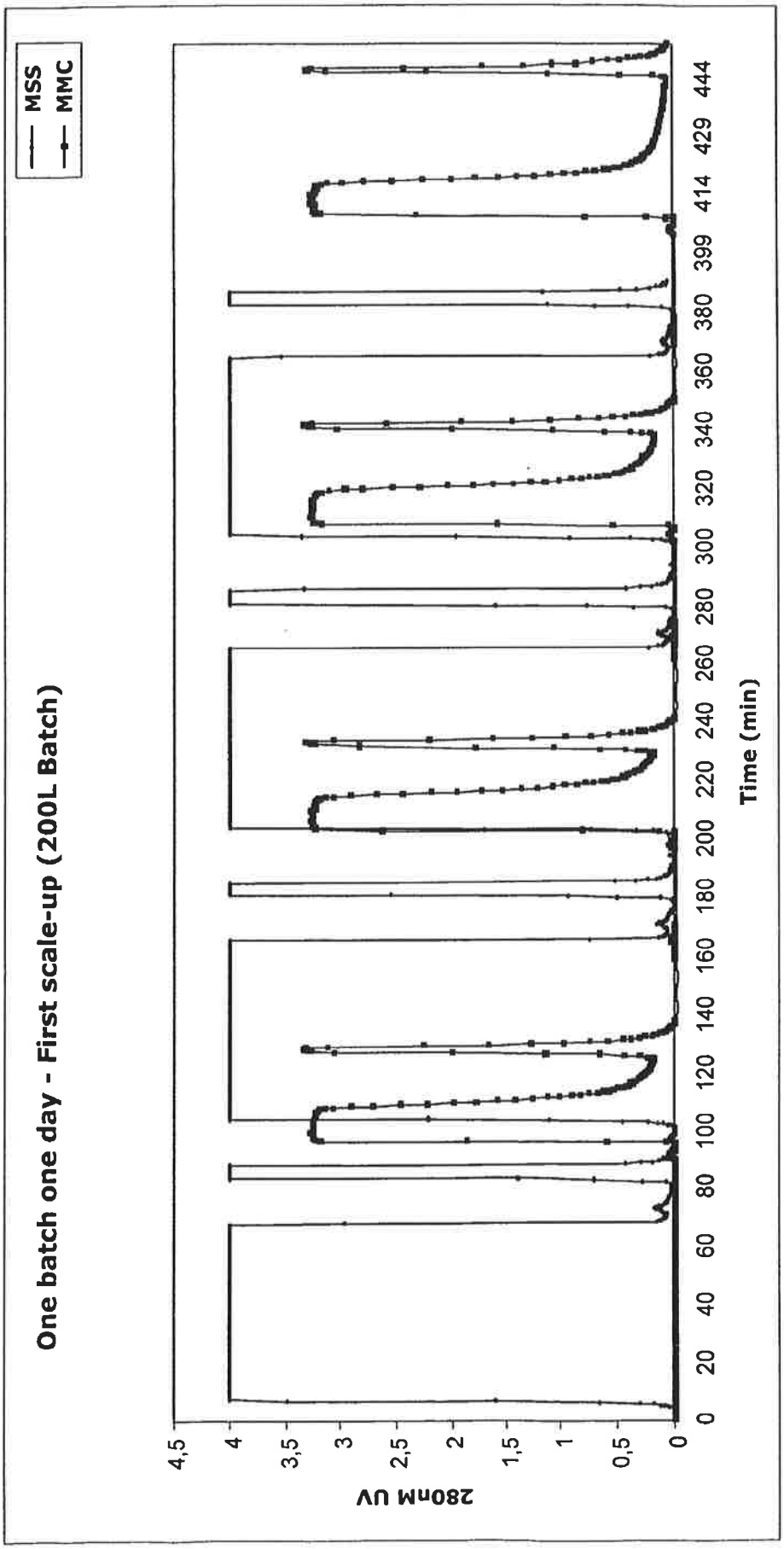
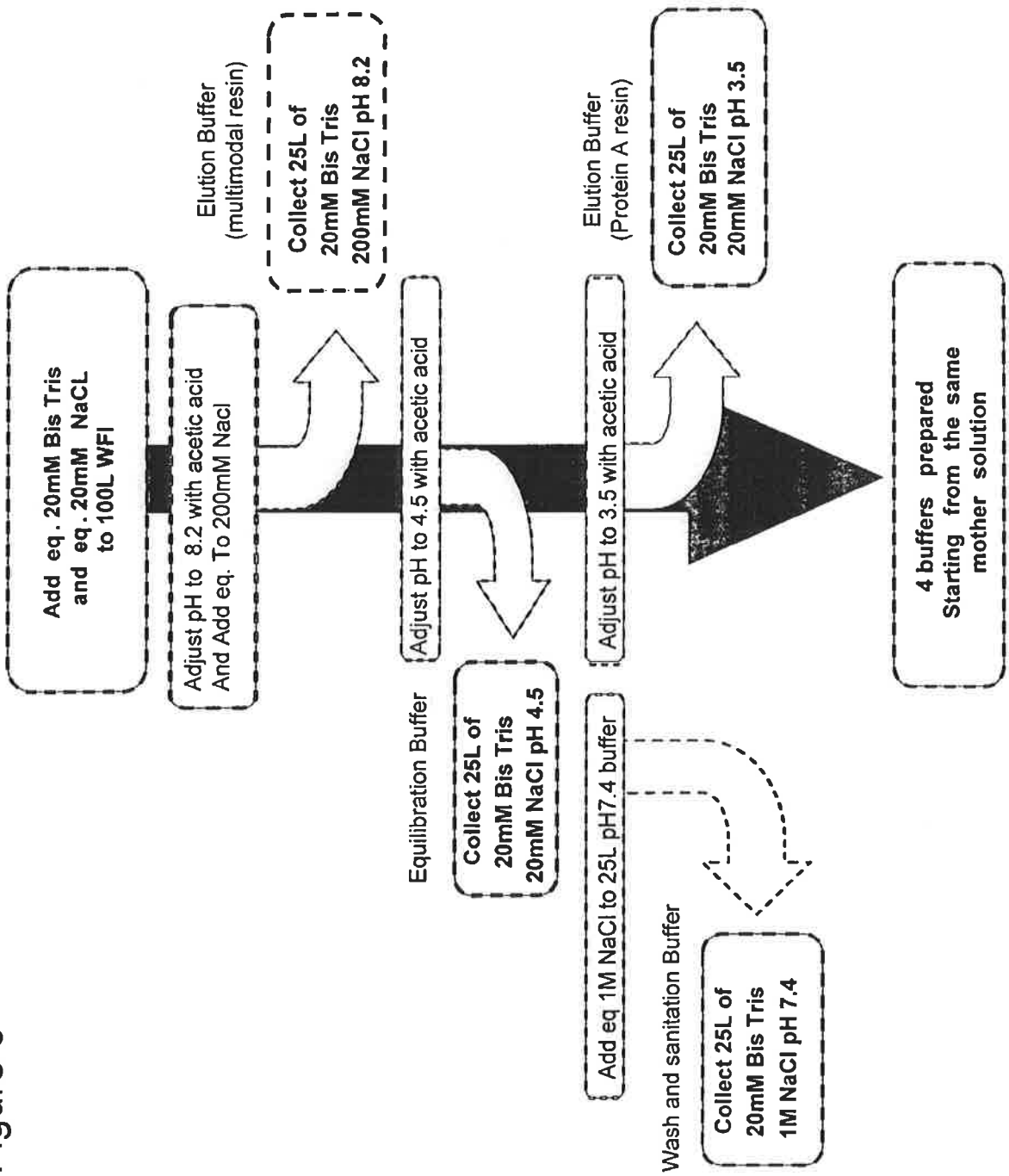

One batch one day - First scale-up (200L Batch)
MSS
MMC
280nM UV
4,5
4
3,5
3
2,5
2
1,5
1
0,5
0
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 399 414 429 444
Time (min)

Figure 6


Add eq. 20mM Bis Tris and eq. 20mM NaCL to 100L WFI
Adjust pH to 8.2 with acetic acid And Add eq. To 200mM Nacl
Elution Buffer (multimodal resin)
Collect 25L of 20mM Bis Tris 200mM NaCl pH 8.2
Adjust pH to 4.5 with acetic acid
Equilibration Buffer
Collect 25L of 20mM Bis Tris 20mM NaCl pH 4.5
Adjust pH to 3.5 with acetic acid
Elution Buffer (Protein A resin)
Collect 25L of 20mM Bis Tris 20mM NaCl pH 3.5
Add eq 1M NaCl to 25L pH7.4 buffer
Wash and sanitation Buffer
Collect 25L of 20mM Bis Tris 1M NaCl pH 7.4
4 buffers prepared Starting from the same mother solution