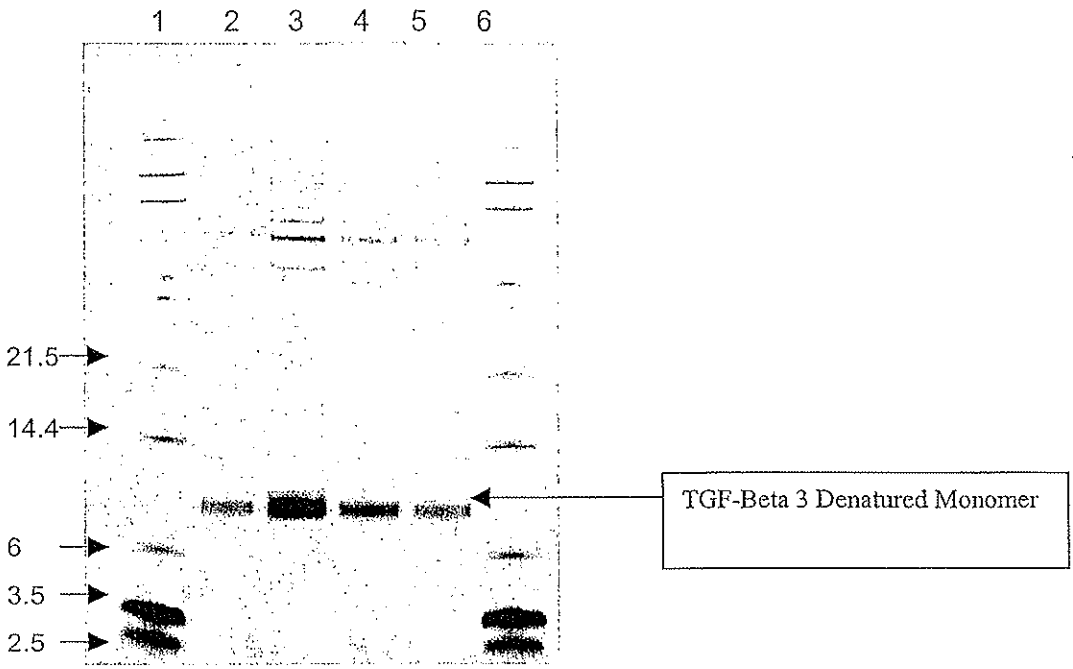
FIG. 1



Lane	Sample
1	Mark 12 Standard
2	Clone1 3hr Post-Induction (3μL)
3	Clone 2 3hr Post-Induction (3μL)
4	Clone 3 3hr Post-Induction (3μL)
5	Clone 4 3hr Post-Induction (3μL)
6	Mark 12 standard

FIG. 2

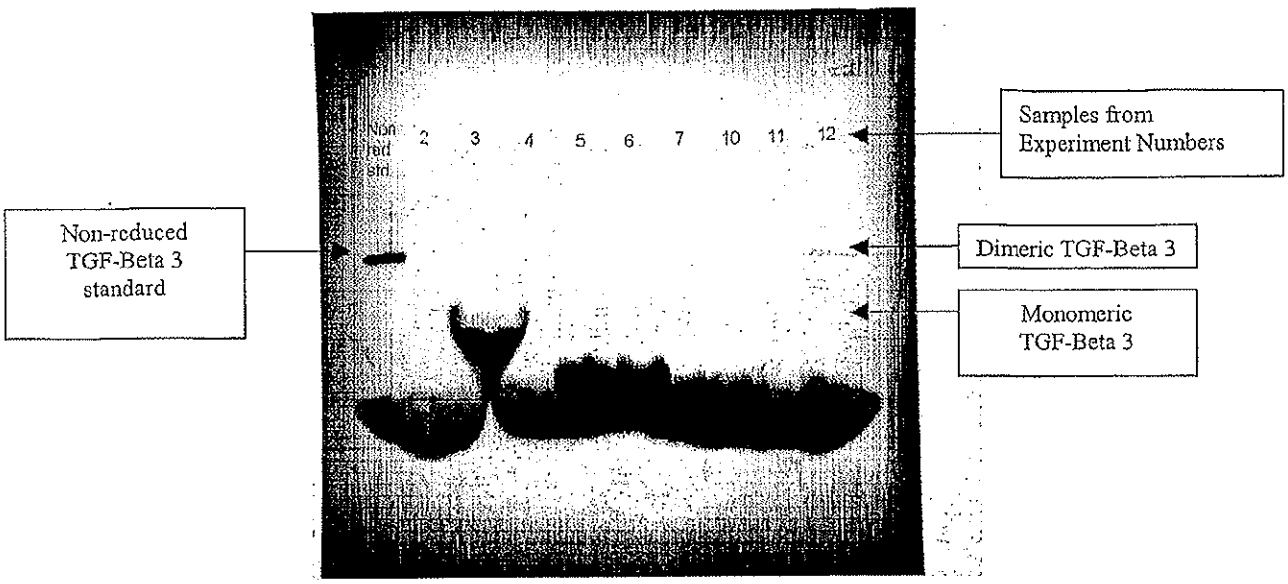

Non red std
2
3
4
5
6
7
10
11
12
Samples from Experiment Numbers
Non-reduced TGF-Beta 3 standard
Dimeric TGF-Beta 3
Monomeric TGF-Beta 3

FIG. 3

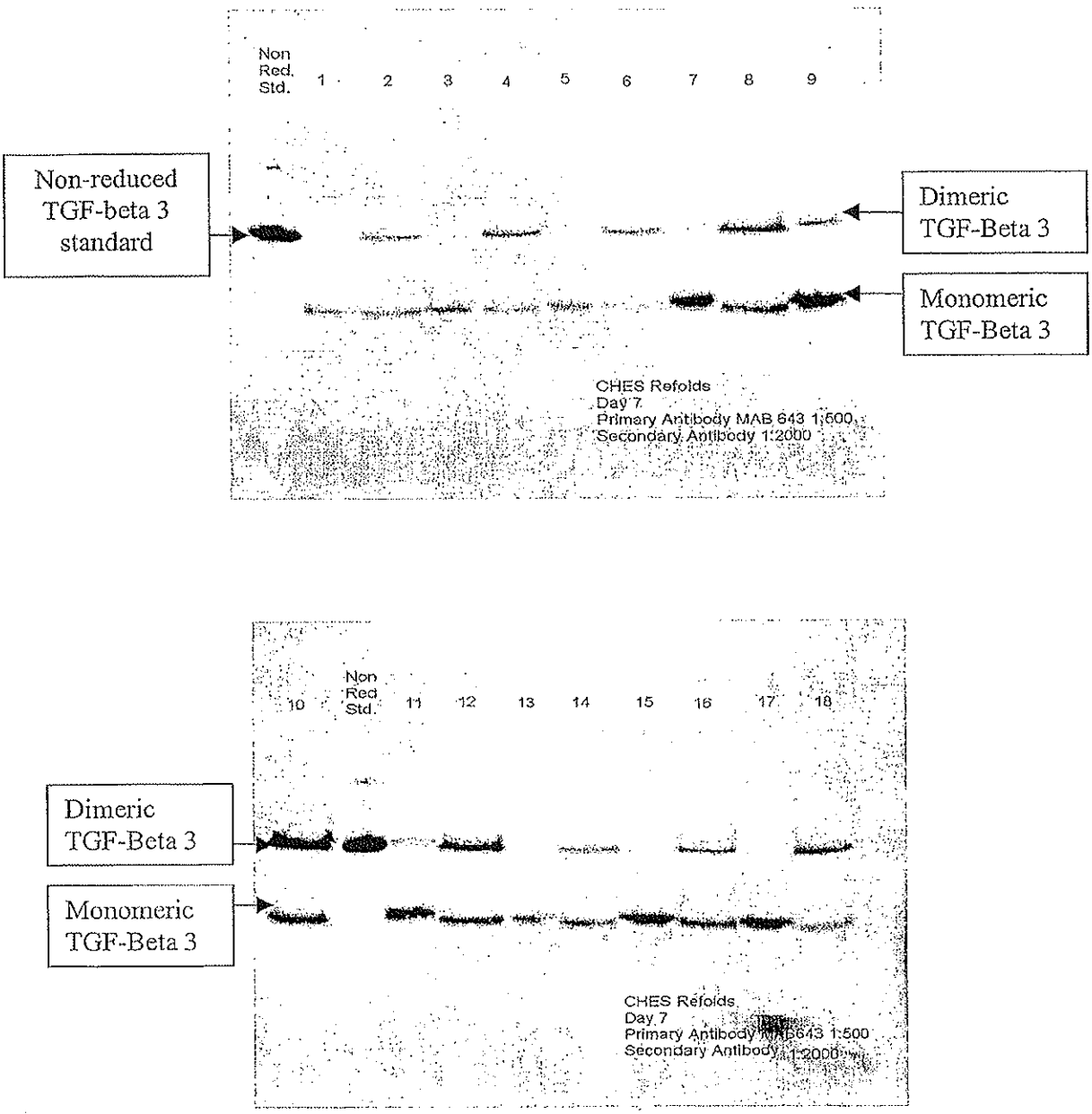

Non Red. Std.
1
2
3
4
5
6
7
8
9
Non-reduced TGF-beta 3 standard
Dimeric TGF-Beta 3
Monomeric TGF-Beta 3
CHES Refolds
Day 7
Primary Antibody MAB 643 1:500
Secondary Antibody 1:2000
Non Red Std.
10
11
12
13
14
15
16
17
18
Dimeric TGF-Beta 3
Monomeric TGF-Beta 3
CHES Refolds
Day 7
Primary Antibody MAB643 1:500
Secondary Antibody 1:2000

FIG. 4

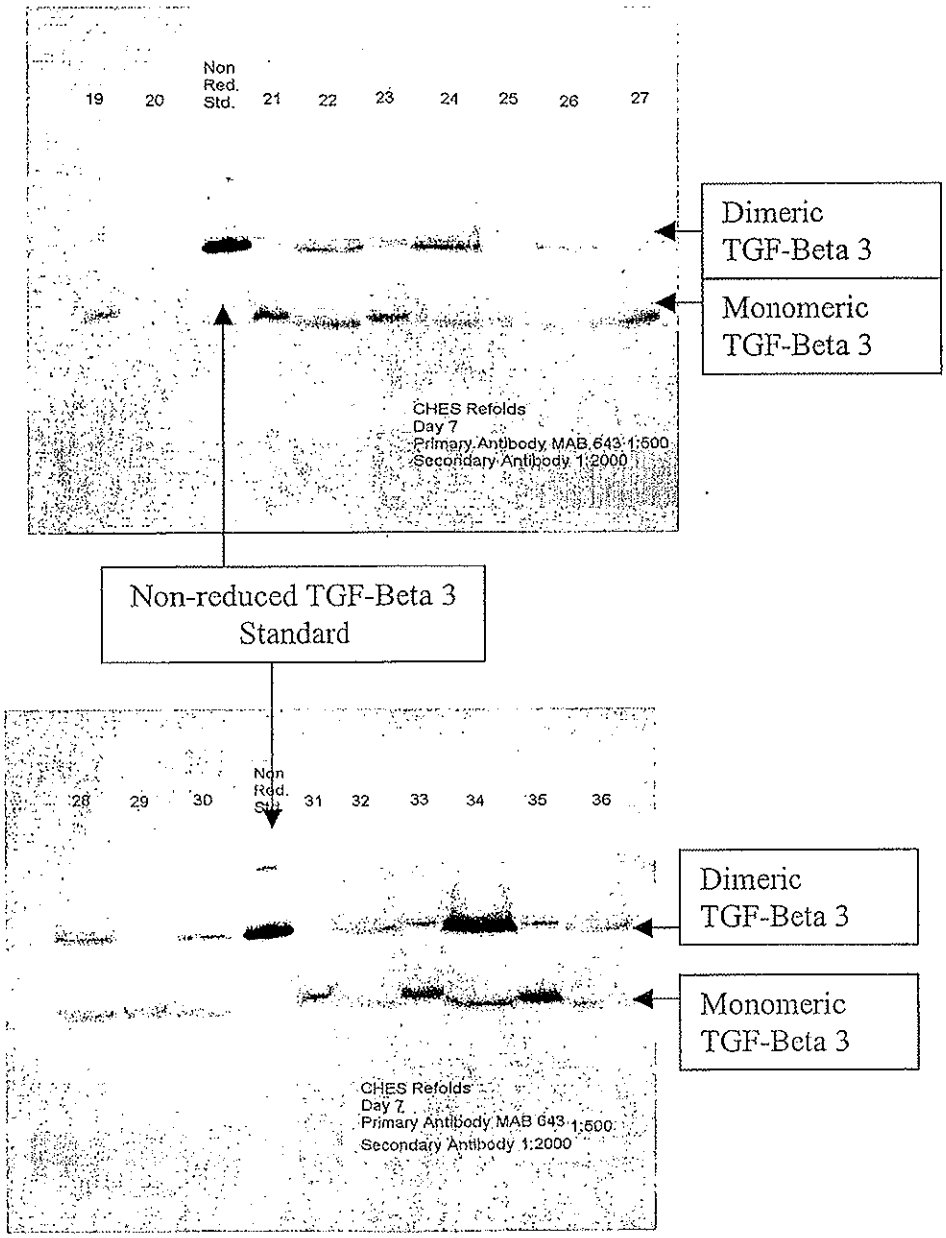
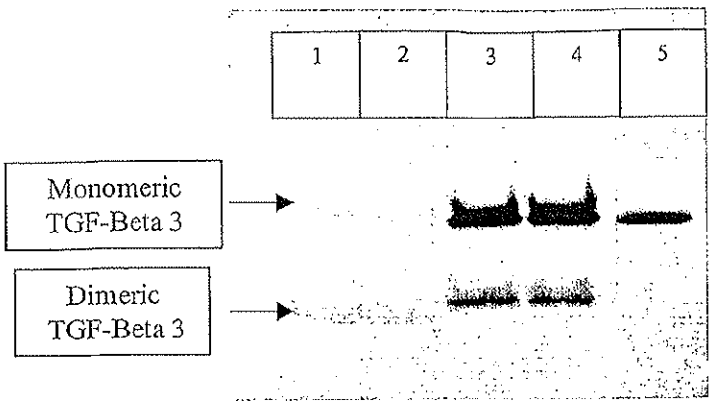
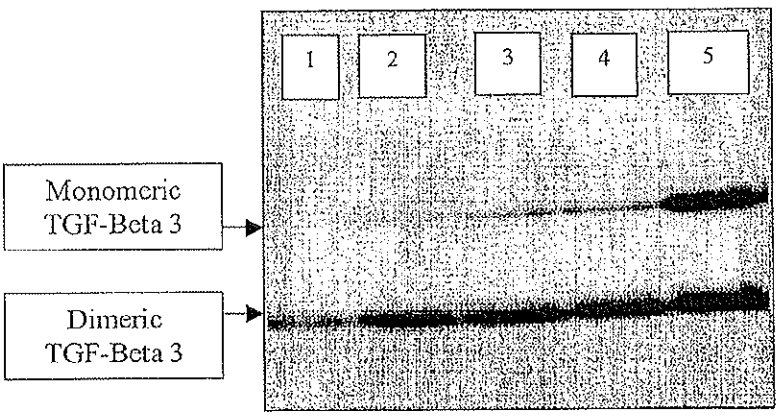

Non Red. Std.
19
20
21
22
23
24
25
26
27
Dimeric TGF-Beta 3
Monomeric TGF-Beta 3
CHES Refolds
Day 7
Primary Antibody MAB 643 1:500
Secondary Antibody 1:2000
Non-reduced TGF-Beta 3 Standard
28
29
30
Non Red. Std.
31
32
33
34
35
36
Dimeric TGF-Beta 3
Monomeric TGF-Beta 3
CHES Refolds
Day 7
Primary Antibody MAB 643 1:500
Secondary Antibody 1:2000

FIG. 5



Lane	Sample
1	Day 0 Re-fold
2	Day 1 re-fold
3	Day 2 re-fold
4	Day 3 re-fold
5	TGF-Beta 3 Standard

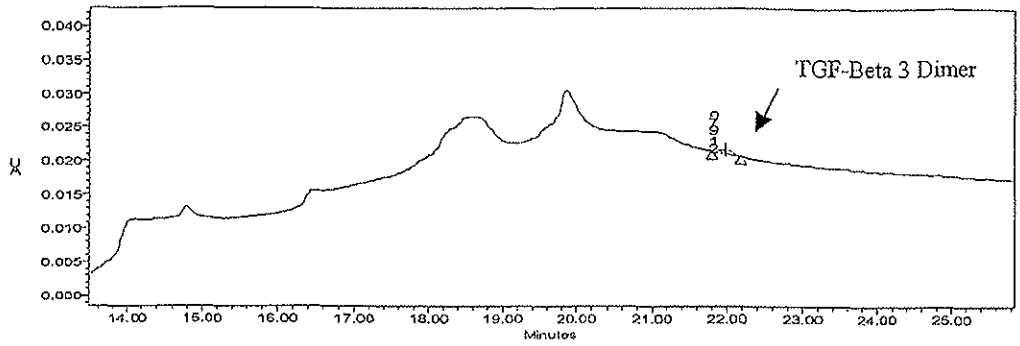
FIG. 6



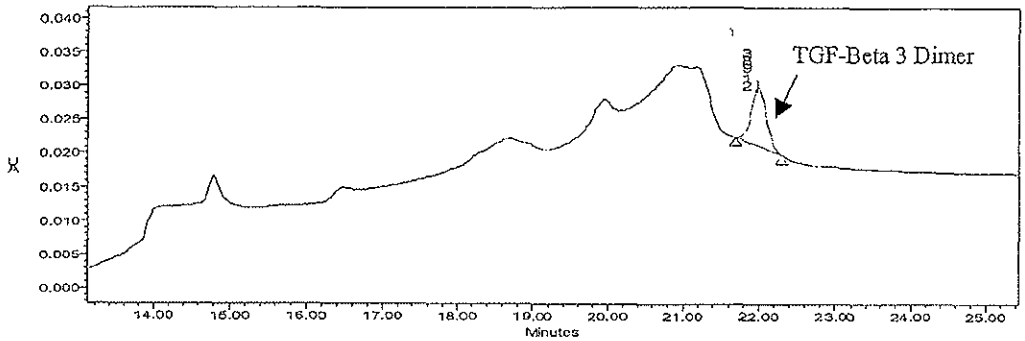
Lane	Sample
1	Day 1 re-fold
2	Day 2 re-fold
3	Day 3 re-fold
4	Day 4 re-fold
5	Day 7 re-fold

FIG. 7

(A)


TGF-Beta 3 Dimer
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00 25.00
Minutes

(B)


TGF-Beta 3 Dimer
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00 25.00
Minutes

(C)

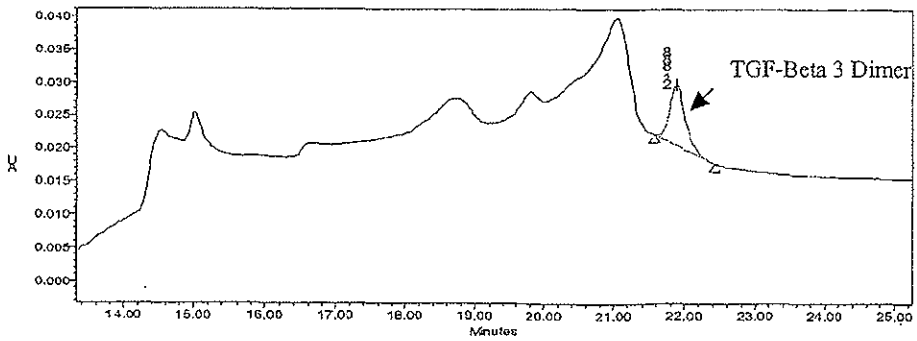

TGF-Beta 3 Dimer
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
14.00 15.00 16.00 17.00 18.00 19.00 20.00 21.00 22.00 23.00 24.00 25.00
Minutes

FIG. 8

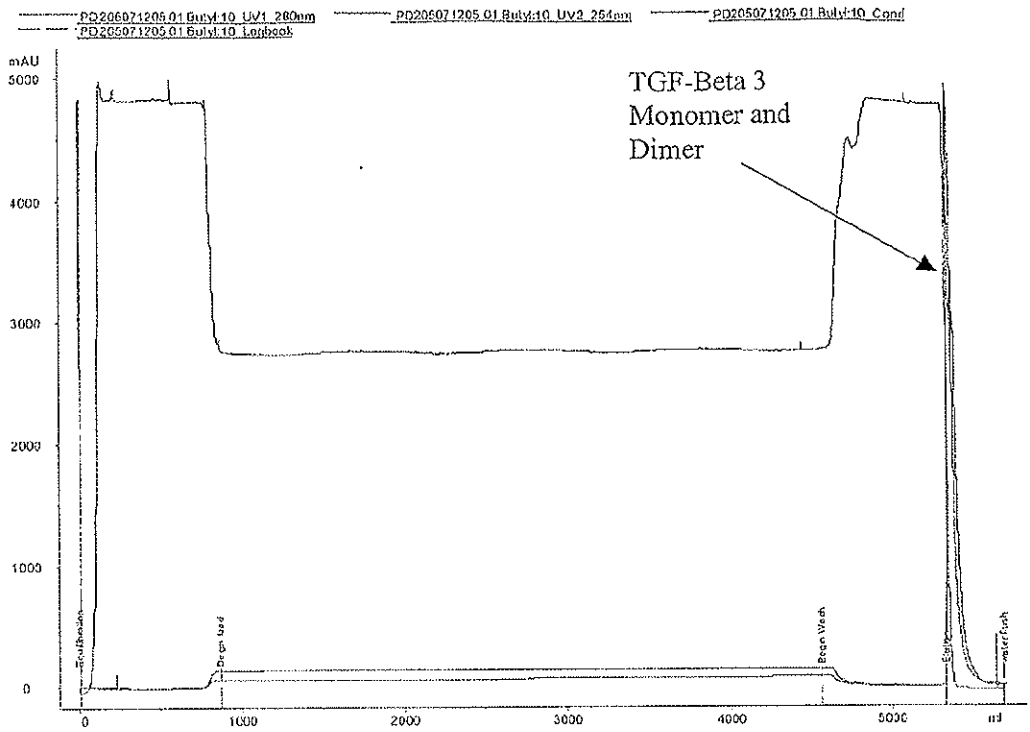

PD20507205.01.Butyl:10_UV1_280nm
PD20507205.01.Butyl:10_UV3_254nm
PD20507205.01.Butyl:10_Cond
PD20507205.01.Butyl:10_Logbook
mAU
5000
4000
3000
2000
1000
0
TGF-Beta 3
Monomer and
Dimer
Equilibration
Begin load
Begin Wash
Elute
Water Wash
0
1000
2000
3000
4000
5000
ml

FIG. 9

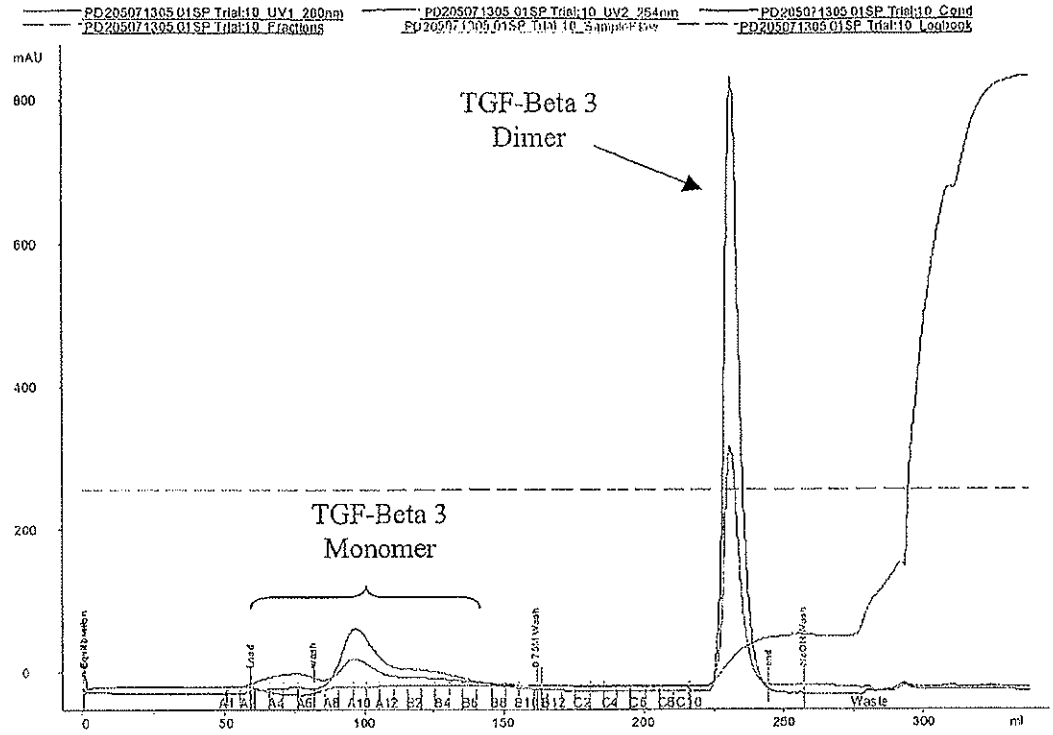

PD20507 1305 01SP Trial:10_UV1_280nm
PD20507 1305 01SP Trial:10_UV2_254nm
PD20507 1305 01SP Trial:10_Cond
PD20507 1305 01SP Trial:10_Fractions
PD20507 1305 01SP Trial:10_SampleFlow
PD20507 1305 01SP Trial:10_Logbook
mAU
800
600
400
200
0
TGF-Beta 3
Dimer
TGF-Beta 3
Monomer
Load
Wash
0.75M Wash
Waste
0
50
100
150
200
250
300
ml

FIG 10

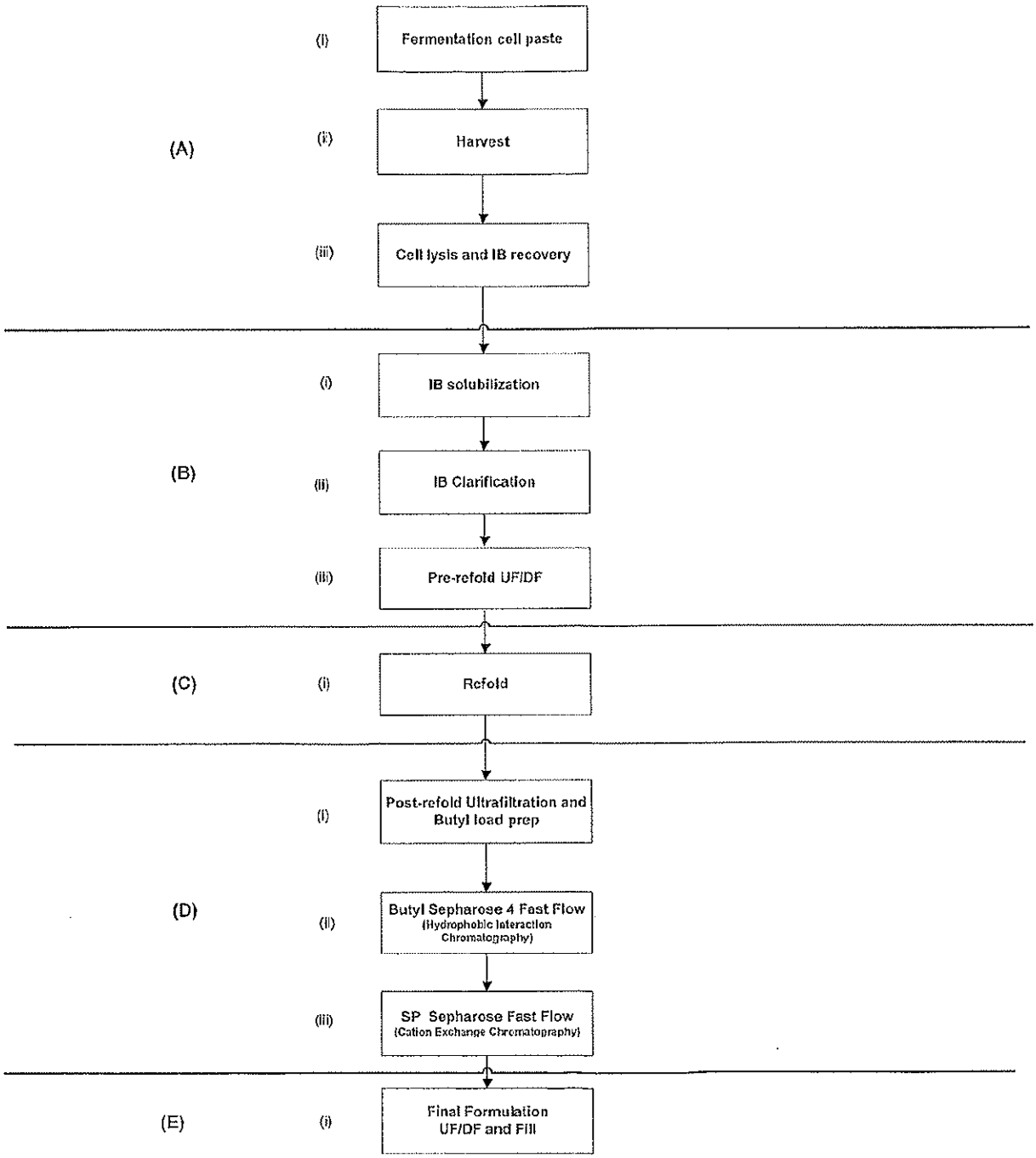

(A)
(i) Fermentation cell paste
(ii) Harvest
(iii) Cell lysis and IB recovery
(B)
(i) IB solubilization
(ii) IB Clarification
(iii) Pre-refold UF/DF
(C)
(i) Refold
(D)
(i) Post-refold Ultrafiltration and Butyl load prep
(ii) Butyl Sepharose 4 Fast Flow (Hydrophobic Interaction Chromatography)
(iii) SP Sepharose Fast Flow (Cation Exchange Chromatography)
(E)
(i) Final Formulation UF/DF and Fill

FIG 11

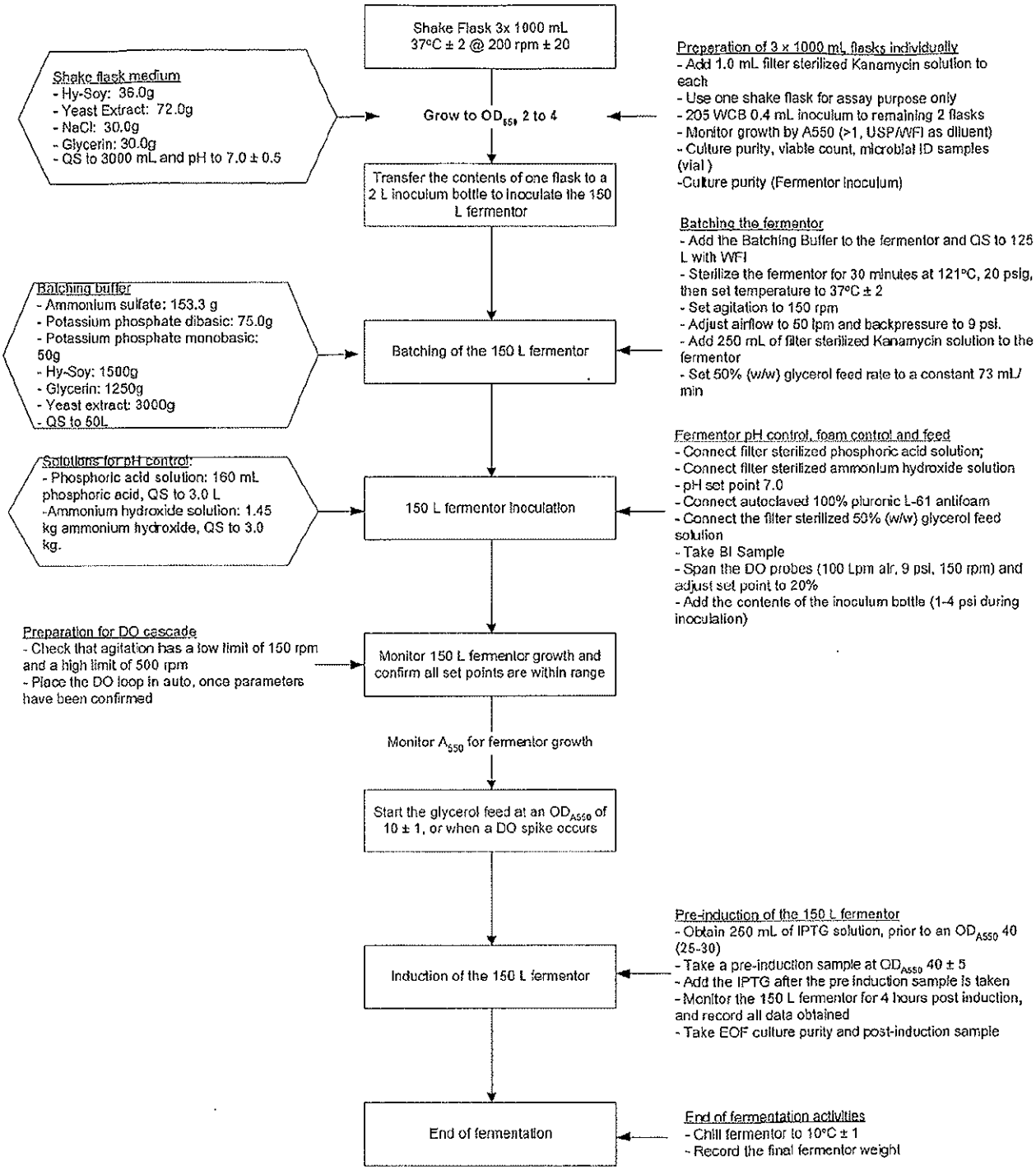

Shake Flask 3x 1000 mL
37°C ± 2 @ 200 rpm ± 20
Shake flask medium
- Hy-Soy: 36.0g
- Yeast Extract: 72.0g
- NaCl: 30.0g
- Glycerin: 30.0g
- QS to 3000 mL and pH to 7.0 ± 0.5
Grow to OD650 2 to 4
Preparation of 3 x 1000 mL flasks individually
- Add 1.0 mL filter sterilized Kanamycin solution to each
- Use one shake flask for assay purpose only
- 205 WCB 0.4 mL inoculum to remaining 2 flasks
- Monitor growth by A550 (>1, USP/WFI as diluent)
- Culture purity, viable count, microbial ID samples (vial)
-Culture purity (Fermentor Inoculum)
Transfer the contents of one flask to a 2 L inoculum bottle to inoculate the 150 L fermentor
Batching buffer
- Ammonium sulfate: 153.3 g
- Potassium phosphate dibasic: 75.0g
- Potassium phosphate monobasic: 50g
- Hy-Soy: 1500g
- Glycerin: 1250g
- Yeast extract: 3000g
- QS to 50L
Batching of the 150 L fermentor
Batching the fermentor
- Add the Batching Buffer to the fermentor and QS to 125 L with WFI
- Sterilize the fermentor for 30 minutes at 121°C, 20 psig, then set temperature to 37°C ± 2
- Set agitation to 150 rpm
- Adjust airflow to 50 lpm and backpressure to 9 psi.
- Add 250 mL of filter sterilized Kanamycin solution to the fermentor
- Set 50% (w/w) glycerol feed rate to a constant 73 mL/min
Solutions for pH control:
- Phosphoric acid solution: 160 mL phosphoric acid, QS to 3.0 L
-Ammonium hydroxide solution: 1.45 kg ammonium hydroxide, QS to 3.0 kg.
150 L fermentor inoculation
Fermentor pH control, foam control and feed
- Connect filter sterilized phosphoric acid solution;
- Connect filter sterilized ammonium hydroxide solution
- pH set point 7.0
- Connect autoclaved 100% pluronic L-61 antifoam
- Connect the filter sterilized 50% (w/w) glycerol feed solution
- Take BI Sample
- Span the DO probes (100 Lpm air, 9 psi, 150 rpm) and adjust set point to 20%
- Add the contents of the inoculum bottle (1-4 psi during inoculation)
Preparation for DO cascade
- Check that agitation has a low limit of 150 rpm and a high limit of 500 rpm
- Place the DO loop in auto, once parameters have been confirmed
Monitor 150 L fermentor growth and confirm all set points are within range
Monitor A550 for fermentor growth
Start the glycerol feed at an OD550 of 10 ± 1, or when a DO spike occurs
Induction of the 150 L fermentor
Pre-induction of the 150 L fermentor
- Obtain 250 mL of IPTG solution, prior to an OD550 40 (25-30)
- Take a pre-induction sample at OD550 40 ± 5
- Add the IPTG after the pre induction sample is taken
- Monitor the 150 L fermentor for 4 hours post induction, and record all data obtained
- Take EOF culture purity and post-induction sample
End of fermentation
End of fermentation activities
- Chill fermentor to 10°C ± 1
- Record the final fermentor weight

FIG 12

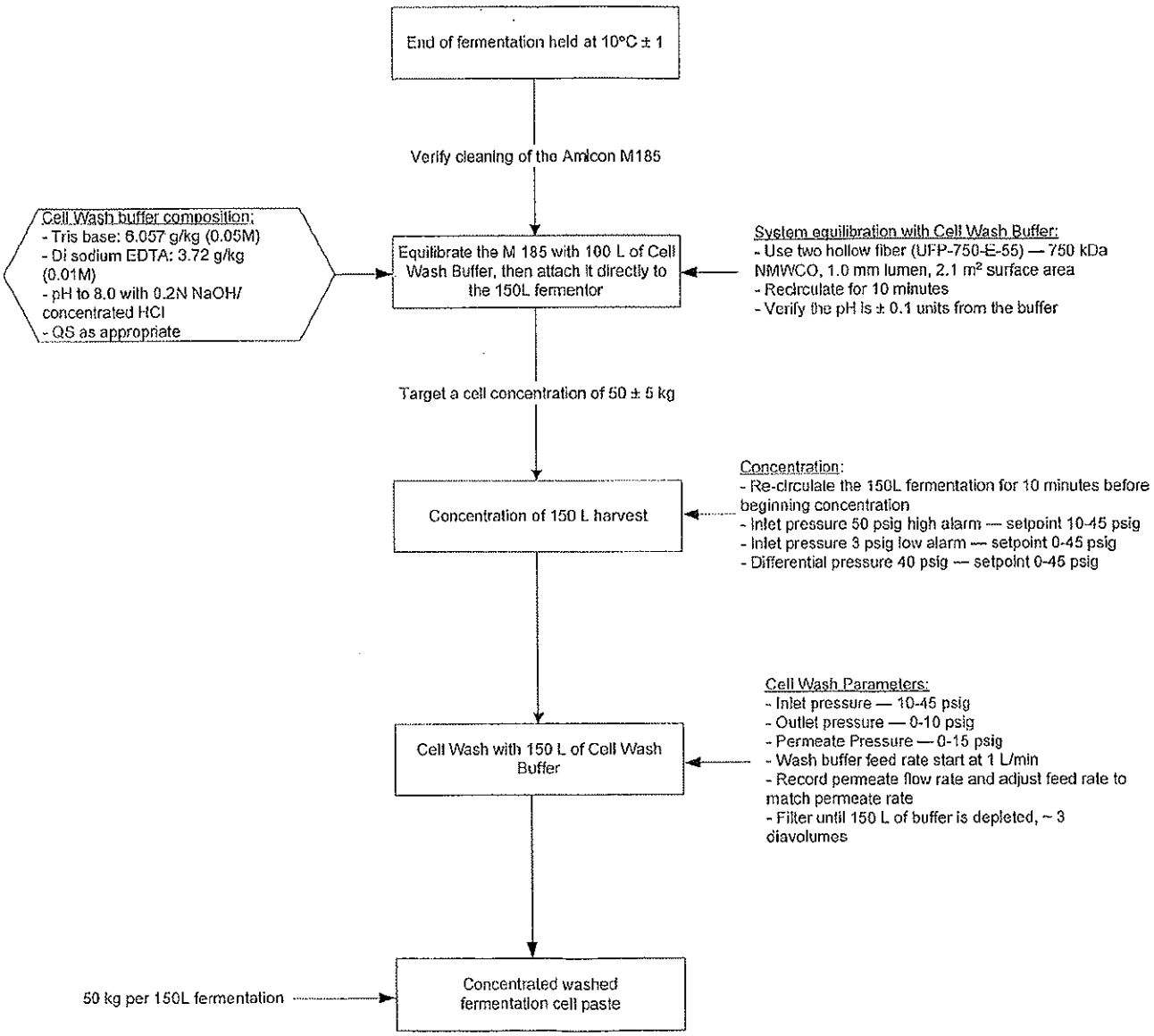

End of fermentation held at 10°C ± 1
Verify cleaning of the Amicon M185
Cell Wash buffer composition:
- Tris base: 6.057 g/kg (0.05M)
- Di sodium EDTA: 3.72 g/kg (0.01M)
- pH to 8.0 with 0.2N NaOH/ concentrated HCl
- QS as appropriate
Equilibrate the M 185 with 100 L of Cell Wash Buffer, then attach it directly to the 150L fermentor
System equilibration with Cell Wash Buffer:
- Use two hollow fiber (UFP-750-E-55) — 750 kDa NMWCO, 1.0 mm lumen, 2.1 m² surface area
- Recirculate for 10 minutes
- Verify the pH is ± 0.1 units from the buffer
Target a cell concentration of 50 ± 5 kg
Concentration of 150 L harvest
Concentration:
- Re-circulate the 150L fermentation for 10 minutes before beginning concentration
- Inlet pressure 50 psig high alarm — setpoint 10-45 psig
- Inlet pressure 3 psig low alarm — setpoint 0-45 psig
- Differential pressure 40 psig — setpoint 0-45 psig
Cell Wash with 150 L of Cell Wash Buffer
Cell Wash Parameters:
- Inlet pressure — 10-45 psig
- Outlet pressure — 0-10 psig
- Permeate Pressure — 0-15 psig
- Wash buffer feed rate start at 1 L/min
- Record permeate flow rate and adjust feed rate to match permeate rate
- Filter until 150 L of buffer is depleted, ~ 3 diavolumes
50 kg per 150L fermentation
Concentrated washed fermentation cell paste

FIG 13

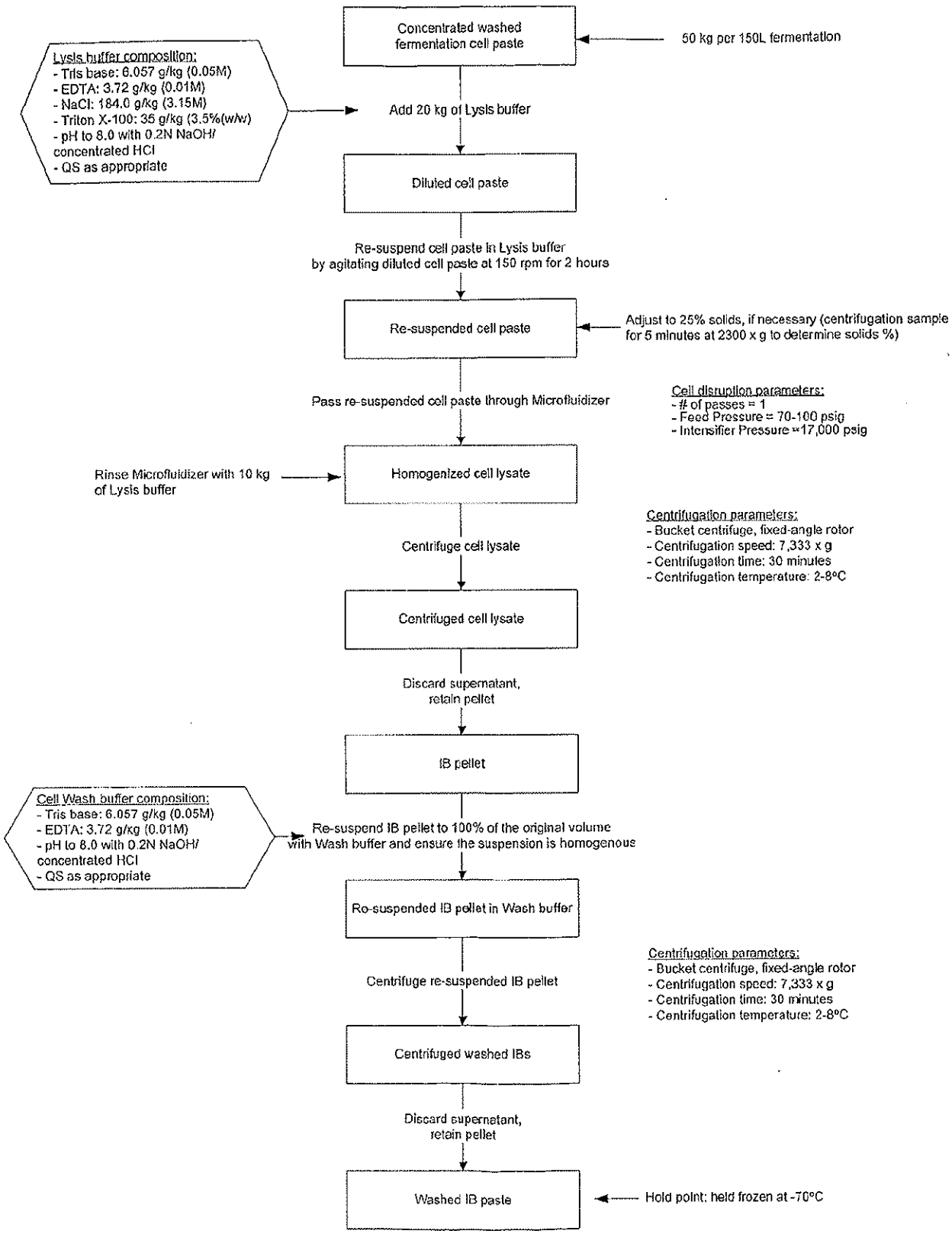

Concentrated washed fermentation cell paste
50 kg per 150L fermentation
Lysis buffer composition:
- Tris base: 6.057 g/kg (0.05M)
- EDTA: 3.72 g/kg (0.01M)
- NaCl: 184.0 g/kg (3.15M)
- Triton X-100: 35 g/kg (3.5%(w/w)
- pH to 8.0 with 0.2N NaOH/ concentrated HCl
- QS as appropriate
Add 20 kg of Lysis buffer
Diluted cell paste
Re-suspend cell paste in Lysis buffer by agitating diluted cell paste at 150 rpm for 2 hours
Re-suspended cell paste
Adjust to 25% solids, if necessary (centrifugation sample for 5 minutes at 2300 x g to determine solids %)
Pass re-suspended cell paste through Microfluidizer
Cell disruption parameters:
- # of passes = 1
- Feed Pressure = 70-100 psig
- Intensifier Pressure ≈17,000 psig
Rinse Microfluidizer with 10 kg of Lysis buffer
Homogenized cell lysate
Centrifuge cell lysate
Centrifugation parameters:
- Bucket centrifuge, fixed-angle rotor
- Centrifugation speed: 7,333 x g
- Centrifugation time: 30 minutes
- Centrifugation temperature: 2-8°C
Centrifuged cell lysate
Discard supernatant, retain pellet
IB pellet
Cell Wash buffer composition:
- Tris base: 6.057 g/kg (0.05M)
- EDTA: 3.72 g/kg (0.01M)
- pH to 8.0 with 0.2N NaOH/ concentrated HCl
- QS as appropriate
Re-suspend IB pellet to 100% of the original volume with Wash buffer and ensure the suspension is homogenous
Re-suspended IB pellet in Wash buffer
Centrifuge re-suspended IB pellet
Centrifugation parameters:
- Bucket centrifuge, fixed-angle rotor
- Centrifugation speed: 7,333 x g
- Centrifugation time: 30 minutes
- Centrifugation temperature: 2-8°C
Centrifuged washed IBs
Discard supernatant, retain pellet
Washed IB paste
Hold point: held frozen at -70°C

FIG 14

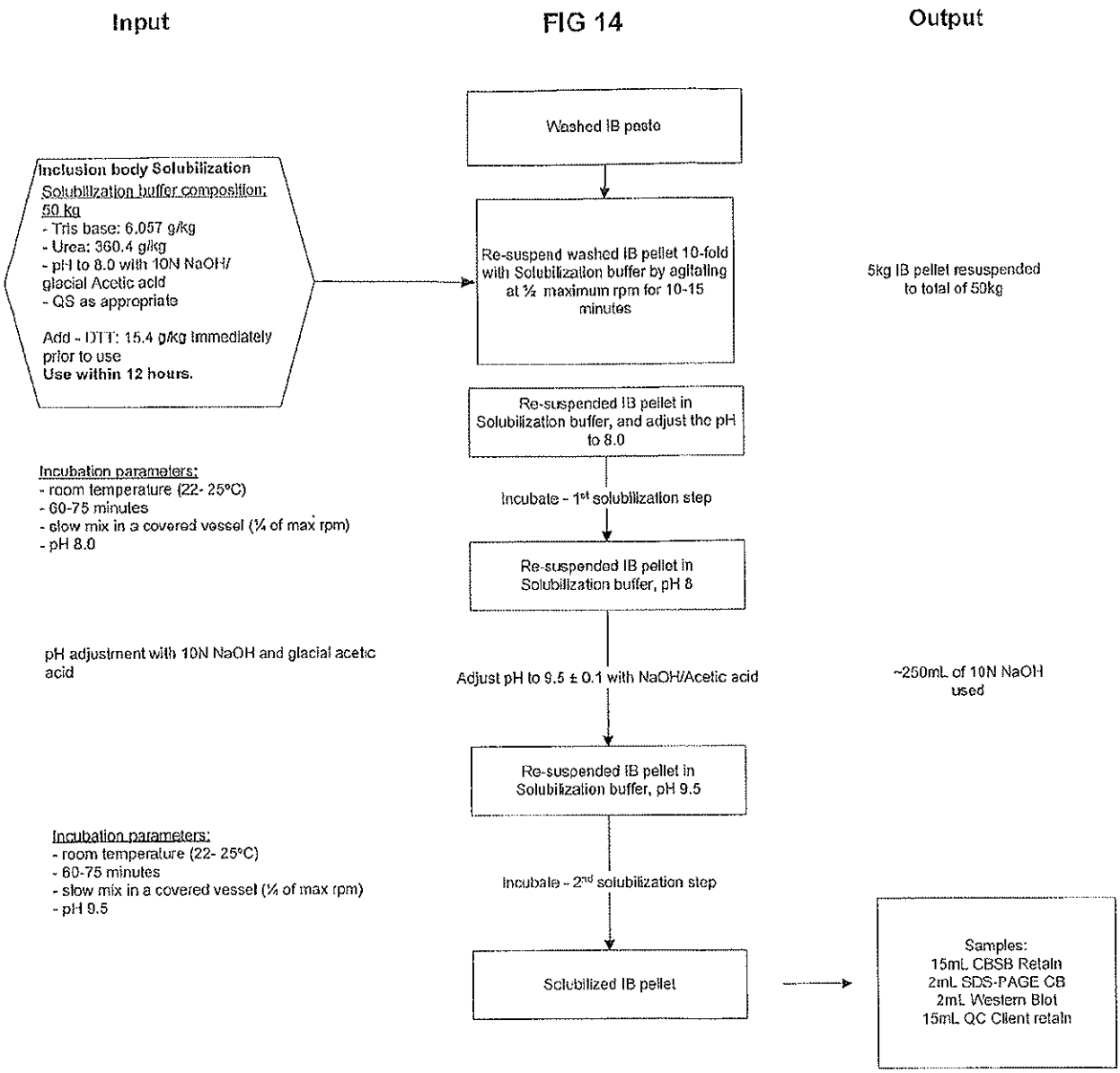
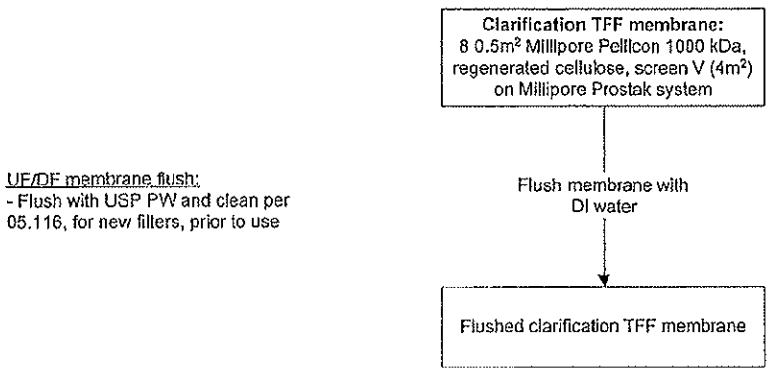

Input
Output
Washed IB paste
Inclusion body Solubilization
Solubilization buffer composition:
50 kg
- Tris base: 6.057 g/kg
- Urea: 360.4 g/kg
- pH to 8.0 with 10N NaOH/ glacial Acetic acid
- QS as appropriate
Add - DTT: 15.4 g/kg immediately prior to use
Use within 12 hours.
Re-suspend washed IB pellet 10-fold with Solubilization buffer by agitating at ⅓ maximum rpm for 10-15 minutes
5kg IB pellet resuspended to total of 50kg
Re-suspended IB pellet in Solubilization buffer, and adjust the pH to 8.0
Incubation parameters:
- room temperature (22- 25°C)
- 60-75 minutes
- slow mix in a covered vessel (¼ of max rpm)
- pH 8.0
Incubate - 1st solubilization step
Re-suspended IB pellet in Solubilization buffer, pH 8
pH adjustment with 10N NaOH and glacial acetic acid
Adjust pH to 9.5 ± 0.1 with NaOH/Acetic acid
~250mL of 10N NaOH used
Re-suspended IB pellet in Solubilization buffer, pH 9.5
Incubation parameters:
- room temperature (22- 25°C)
- 60-75 minutes
- slow mix in a covered vessel (¼ of max rpm)
- pH 9.5
Incubate - 2nd solubilization step
Solubilized IB pellet
Samples:
15mL CBSB Retain
2mL SDS-PAGE CB
2mL Western Blot
15mL QC Client retain

FIG 15

(A)


Clarification TFF membrane:
8 0.5m² Millipore Pellicon 1000 kDa, regenerated cellulose, screen V (4m²) on Millipore Prostak system
Flush membrane with DI water
Flushed clarification TFF membrane
UF/DF membrane flush:
- Flush with USP PW and clean per 05.116, for new filters, prior to use

(B)

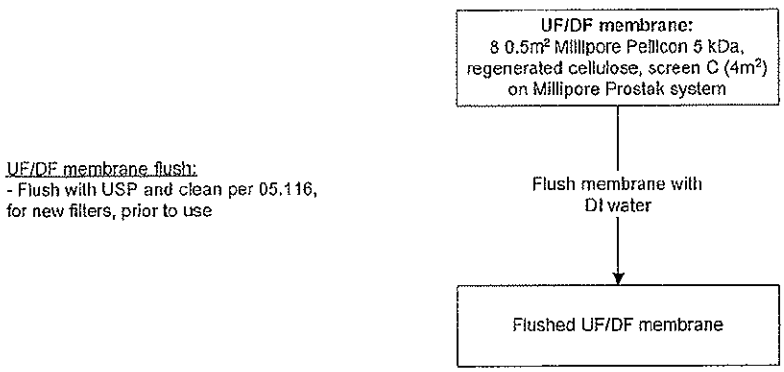

UF/DF membrane:
8 0.5m² Millipore Pellicon 5 kDa, regenerated cellulose, screen C (4m²) on Millipore Prostak system
Flush membrane with DI water
Flushed UF/DF membrane
UF/DF membrane flush:
- Flush with USP and clean per 05.116, for new filters, prior to use

FIG 16A

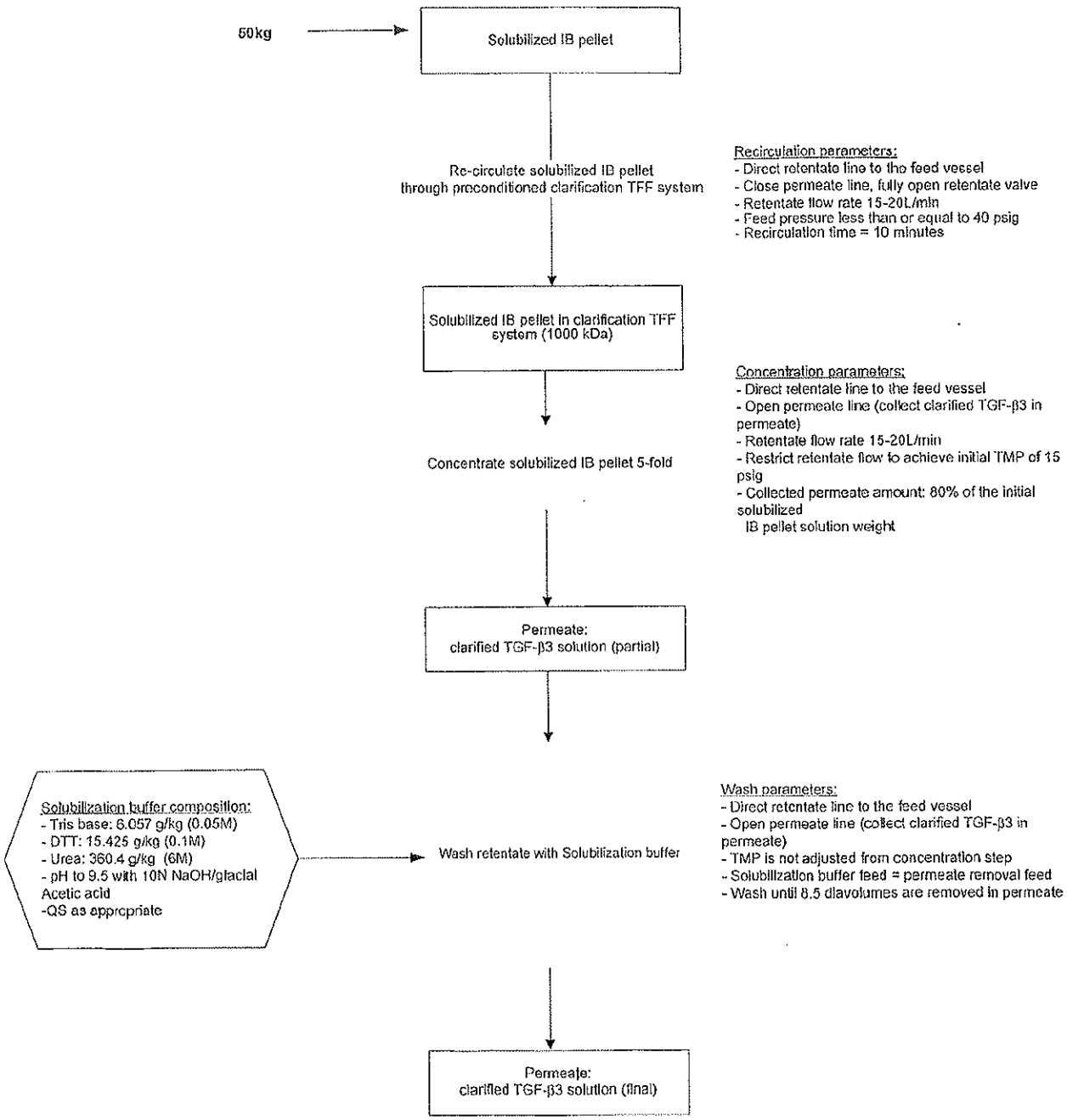

50kg
Solubilized IB pellet
Re-circulate solubilized IB pellet through preconditioned clarification TFF system
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Retentate flow rate 15-20L/min
- Feed pressure less than or equal to 40 psig
- Recirculation time = 10 minutes
Solubilized IB pellet in clarification TFF system (1000 kDa)
Concentrate solubilized IB pellet 5-fold
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (collect clarified TGF-β3 in permeate)
- Retentate flow rate 15-20L/min
- Restrict retentate flow to achieve initial TMP of 15 psig
- Collected permeate amount: 80% of the initial solubilized IB pellet solution weight
Permeate: clarified TGF-β3 solution (partial)
Solubilization buffer composition:
- Tris base: 6.057 g/kg (0.05M)
- DTT: 15.425 g/kg (0.1M)
- Urea: 360.4 g/kg (6M)
- pH to 9.5 with 10N NaOH/glacial Acetic acid
-QS as appropriate
Wash retentate with Solubilization buffer
Wash parameters:
- Direct retentate line to the feed vessel
- Open permeate line (collect clarified TGF-β3 in permeate)
- TMP is not adjusted from concentration step
- Solubilization buffer feed = permeate removal feed
- Wash until 8.5 diavolumes are removed in permeate
Permeate: clarified TGF-β3 solution (final)

FIG 16B

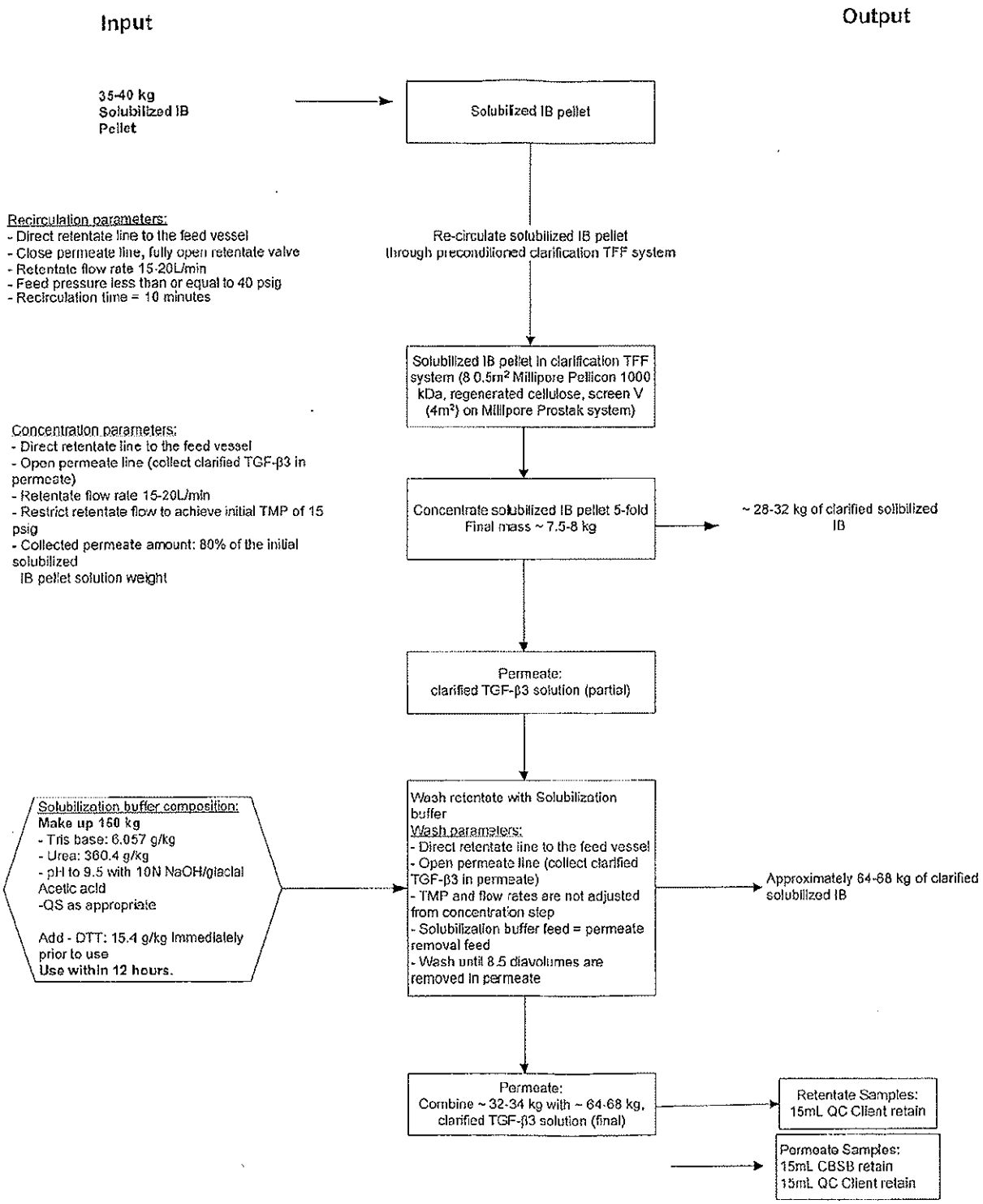

Input
Output
35-40 kg Solubilized IB Pellet
Solubilized IB pellet
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Retentate flow rate 15-20L/min
- Feed pressure less than or equal to 40 psig
- Recirculation time = 10 minutes
Re-circulate solubilized IB pellet through preconditioned clarification TFF system
Solubilized IB pellet in clarification TFF system (8 0.5m² Millipore Pellicon 1000 kDa, regenerated cellulose, screen V (4m²) on Millipore Prostak system)
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (collect clarified TGF-β3 in permeate)
- Retentate flow rate 15-20L/min
- Restrict retentate flow to achieve initial TMP of 15 psig
- Collected permeate amount: 80% of the initial solubilized IB pellet solution weight
Concentrate solubilized IB pellet 5-fold Final mass ~ 7.5-8 kg
~ 28-32 kg of clarified solibilized IB
Permeate: clarified TGF-β3 solution (partial)
Solubilization buffer composition:
Make up 150 kg
- Tris base: 6.057 g/kg
- Urea: 360.4 g/kg
- pH to 9.5 with 10N NaOH/glacial Acetic acid
-QS as appropriate
Add - DTT: 15.4 g/kg Immediately prior to use
Use within 12 hours.
Wash retentate with Solubilization buffer
Wash parameters:
- Direct retentate line to the feed vessel
- Open permeate line (collect clarified TGF-β3 in permeate)
- TMP and flow rates are not adjusted from concentration step
- Solubilization buffer feed = permeate removal feed
- Wash until 8.5 diavolumes are removed in permeate
Approximately 64-68 kg of clarified solubilized IB
Permeate: Combine ~ 32-34 kg with ~ 64-68 kg, clarified TGF-β3 solution (final)
Retentate Samples: 15mL QC Client retain
Permeate Samples: 15mL CBSB retain 15mL QC Client retain

FIG 17A

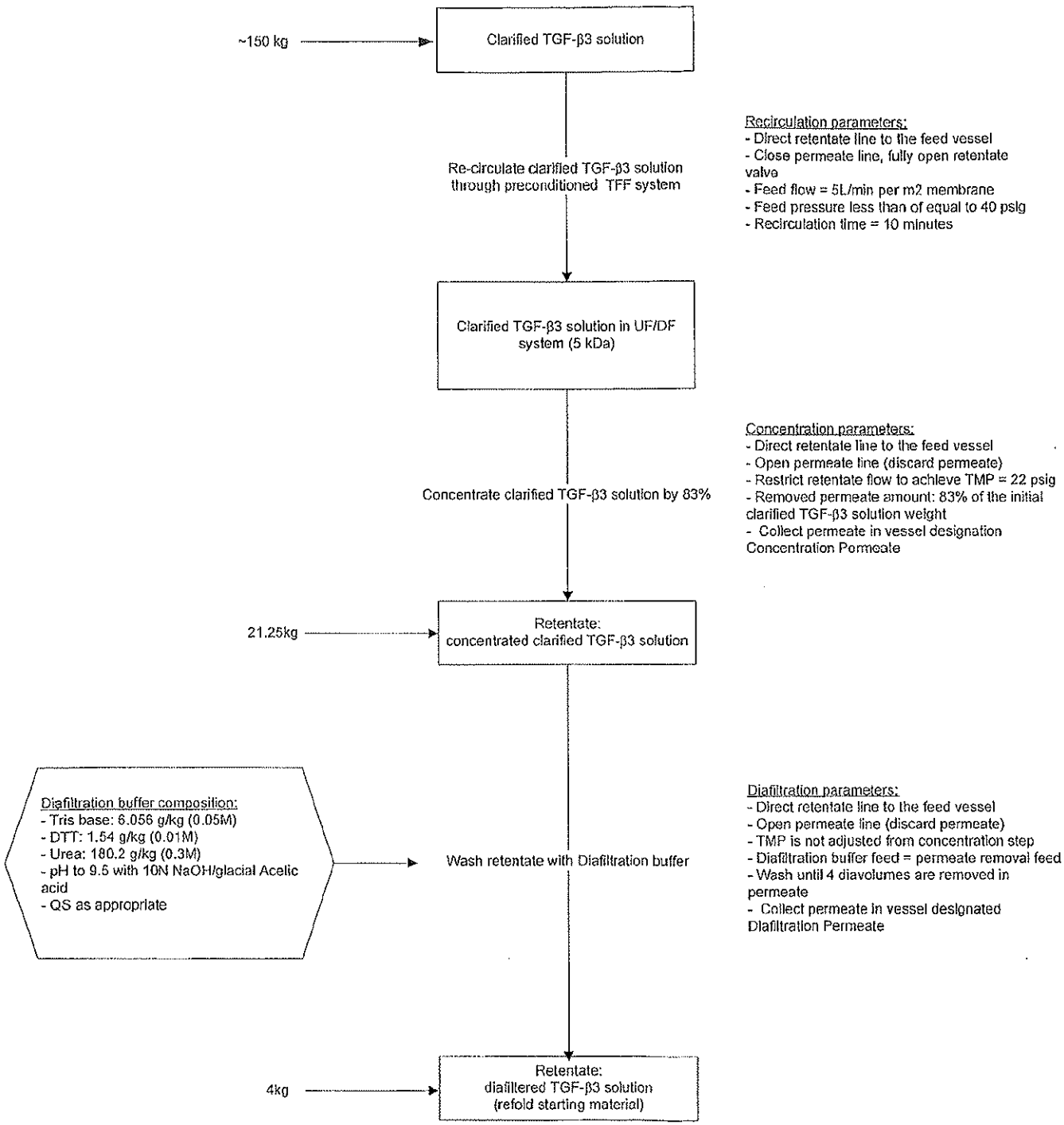

~150 kg
Clarified TGF-β3 solution
Re-circulate clarified TGF-β3 solution through preconditioned TFF system
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Feed flow = 5L/min per m2 membrane
- Feed pressure less than of equal to 40 psig
- Recirculation time = 10 minutes
Clarified TGF-β3 solution in UF/DF system (5 kDa)
Concentrate clarified TGF-β3 solution by 83%
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Restrict retentate flow to achieve TMP = 22 psig
- Removed permeate amount: 83% of the initial clarified TGF-β3 solution weight
- Collect permeate in vessel designation Concentration Permeate
21.25kg
Retentate: concentrated clarified TGF-β3 solution
Diafiltration buffer composition:
- Tris base: 6.056 g/kg (0.05M)
- DTT: 1.54 g/kg (0.01M)
- Urea: 180.2 g/kg (0.3M)
- pH to 9.5 with 10N NaOH/glacial Acelic acid
- QS as appropriate
Wash retentate with Diafiltration buffer
Diafiltration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- TMP is not adjusted from concentration step
- Diafiltration buffer feed = permeate removal feed
- Wash until 4 diavolumes are removed in permeate
- Collect permeate in vessel designated Diafiltration Permeate
4kg
Retentate: diafiltered TGF-β3 solution (refold starting material)

FIG 17B

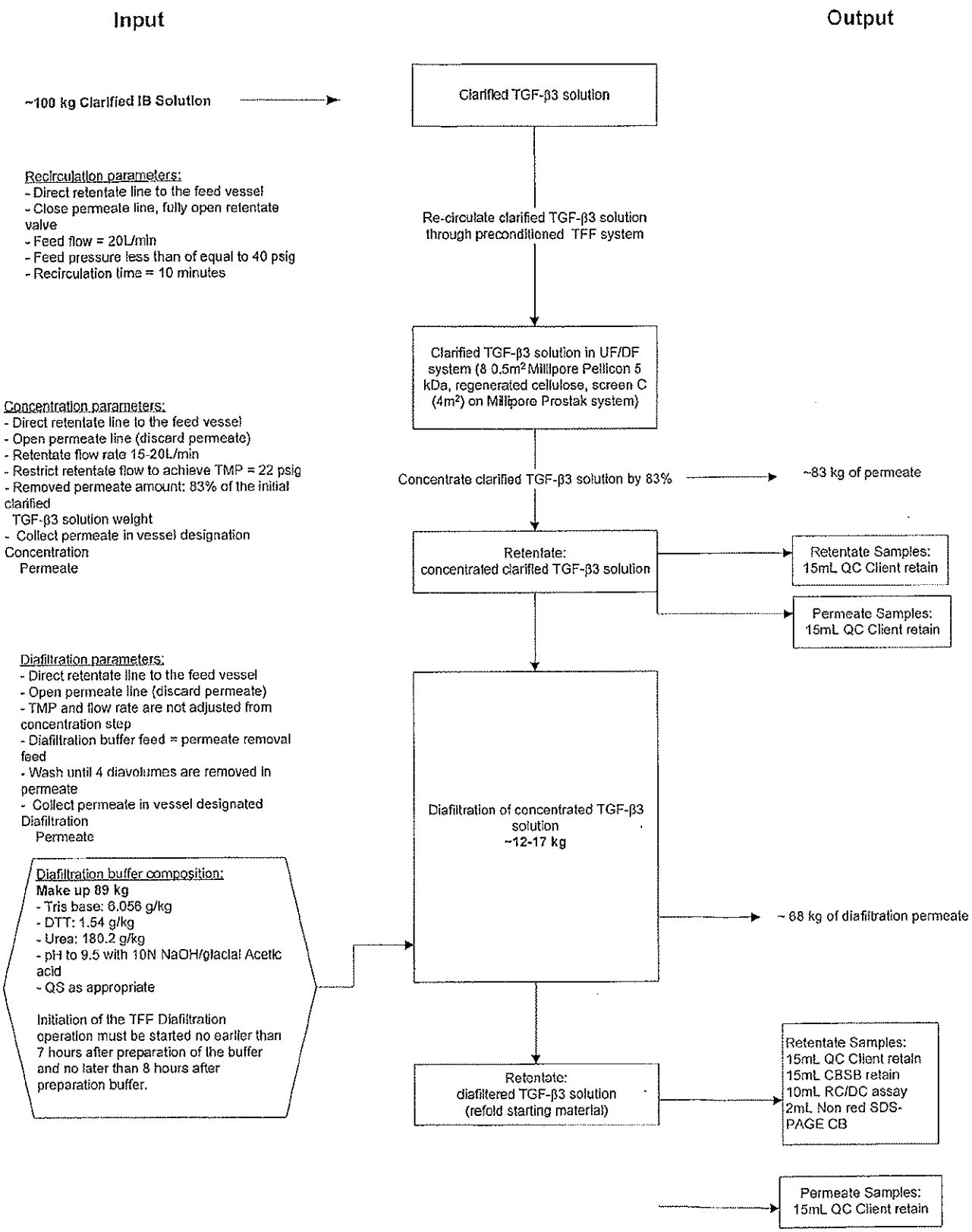

Input
Output
~100 kg Clarified IB Solution
Clarified TGF-β3 solution
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Feed flow = 20L/min
- Feed pressure less than of equal to 40 psig
- Recirculation time = 10 minutes
Re-circulate clarified TGF-β3 solution through preconditioned TFF system
Clarified TGF-β3 solution in UF/DF system (8 0.5m² Millipore Pellicon 5 kDa, regenerated cellulose, screen C (4m²) on Millipore Prostak system)
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Retentate flow rate 15-20L/min
- Restrict retentate flow to achieve TMP = 22 psig
- Removed permeate amount: 83% of the initial clarified
TGF-β3 solution weight
- Collect permeate in vessel designation
Concentration
Permeate
Concentrate clarified TGF-β3 solution by 83%
~83 kg of permeate
Retentate: concentrated clarified TGF-β3 solution
Retentate Samples: 15mL QC Client retain
Permeate Samples: 15mL QC Client retain
Diafiltration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- TMP and flow rate are not adjusted from concentration step
- Diafiltration buffer feed = permeate removal feed
- Wash until 4 diavolumes are removed in permeate
- Collect permeate in vessel designated
Diafiltration
Permeate
Diafiltration of concentrated TGF-β3 solution
~12-17 kg
~ 68 kg of diafiltration permeate
Diafiltration buffer composition:
Make up 89 kg
- Tris base: 6.056 g/kg
- DTT: 1.54 g/kg
- Urea: 180.2 g/kg
- pH to 9.5 with 10N NaOH/glacial Acetic acid
- QS as appropriate
Initiation of the TFF Diafiltration operation must be started no earlier than 7 hours after preparation of the buffer and no later than 8 hours after preparation buffer.
Retentate: diafiltered TGF-β3 solution (refold starting material)
Retentate Samples: 15mL QC Client retain 15mL CBSB retain 10mL RC/DC assay 2mL Non red SDS-PAGE CB
Permeate Samples: 15mL QC Client retain

FIG 18A

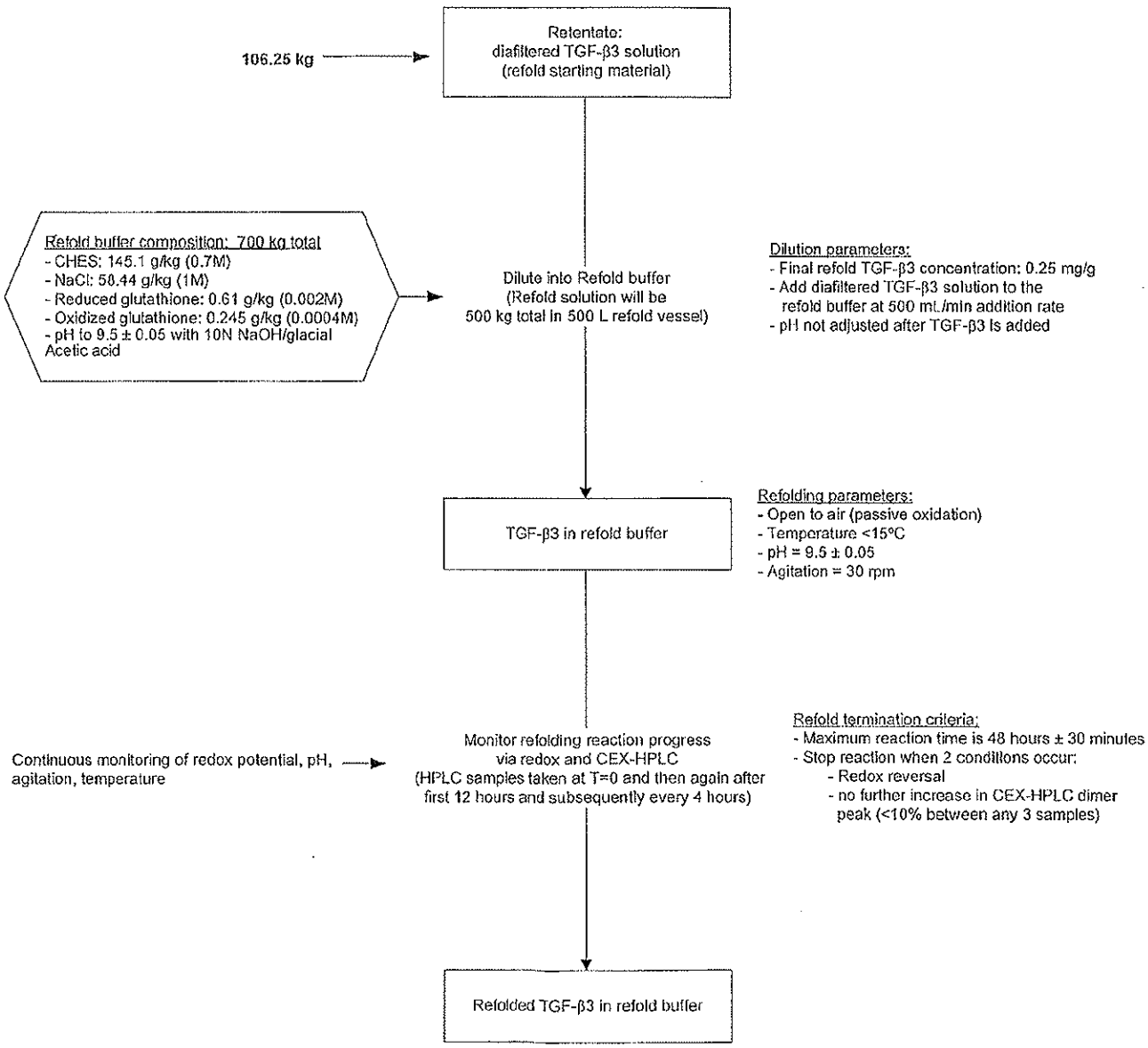

106.25 kg
Retentate:
diafiltered TGF-β3 solution
(refold starting material)
Refold buffer composition: 700 kg total
- CHES: 145.1 g/kg (0.7M)
- NaCl: 58.44 g/kg (1M)
- Reduced glutathione: 0.61 g/kg (0.002M)
- Oxidized glutathione: 0.245 g/kg (0.0004M)
- pH to 9.5 ± 0.05 with 10N NaOH/glacial Acetic acid
Dilute into Refold buffer
(Refold solution will be
500 kg total in 500 L refold vessel)
Dilution parameters:
- Final refold TGF-β3 concentration: 0.25 mg/g
- Add diafiltered TGF-β3 solution to the refold buffer at 500 mL/min addition rate
- pH not adjusted after TGF-β3 is added
TGF-β3 in refold buffer
Refolding parameters:
- Open to air (passive oxidation)
- Temperature <15°C
- pH = 9.5 ± 0.05
- Agitation = 30 rpm
Continuous monitoring of redox potential, pH, agitation, temperature
Monitor refolding reaction progress
via redox and CEX-HPLC
(HPLC samples taken at T=0 and then again after first 12 hours and subsequently every 4 hours)
Refold termination criteria:
- Maximum reaction time is 48 hours ± 30 minutes
- Stop reaction when 2 conditions occur:
- Redox reversal
- no further increase in CEX-HPLC dimer peak (<10% between any 3 samples)
Refolded TGF-β3 in refold buffer

FIG 18B

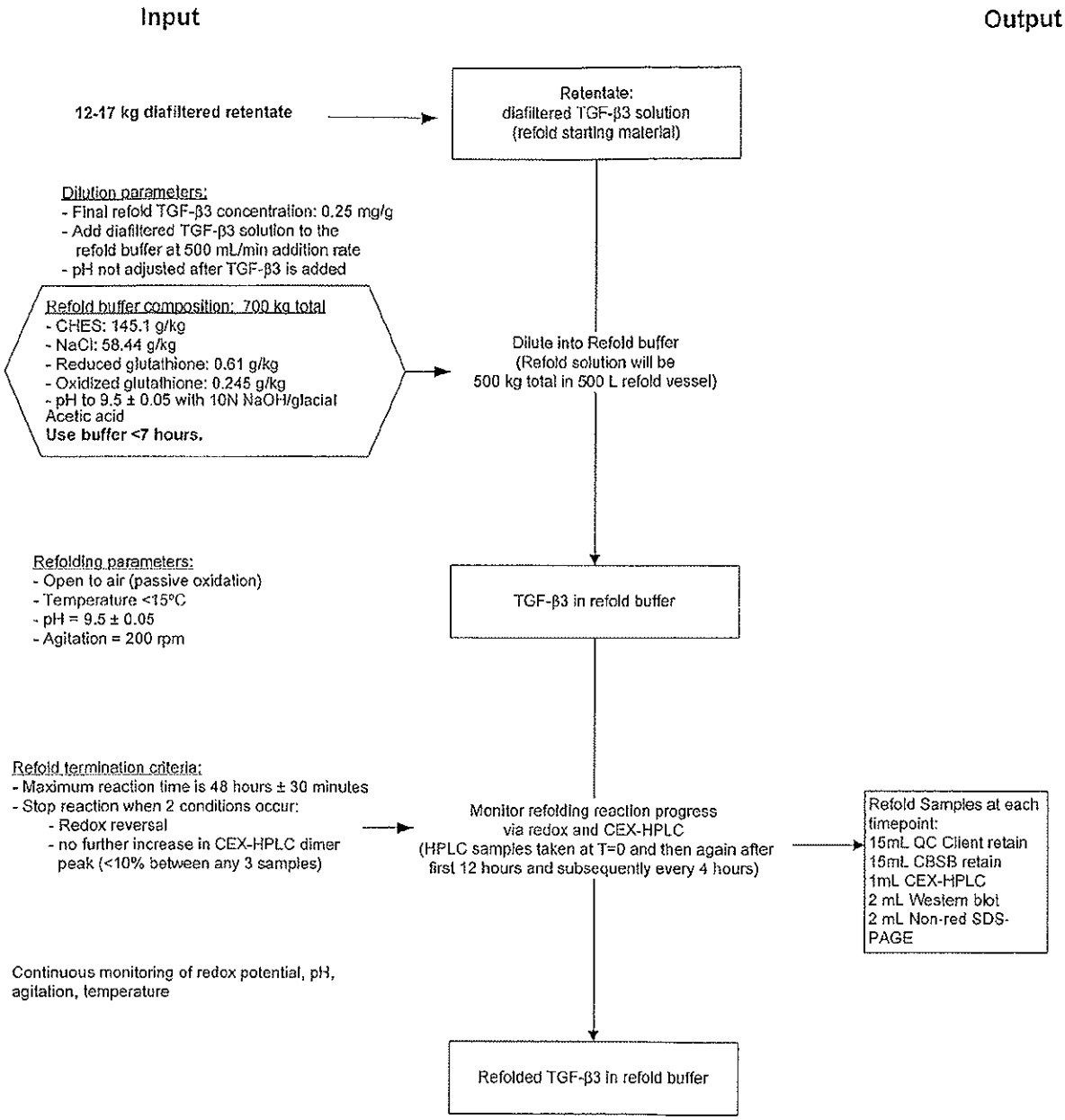

Input
Output
12-17 kg diafiltered retentate
Retentate:
diafiltered TGF-β3 solution
(refold starting material)
Dilution parameters:
- Final refold TGF-β3 concentration: 0.25 mg/g
- Add diafiltered TGF-β3 solution to the refold buffer at 500 mL/min addition rate
- pH not adjusted after TGF-β3 is added
Refold buffer composition: 700 kg total
- CHES: 145.1 g/kg
- NaCl: 58.44 g/kg
- Reduced glutathione: 0.61 g/kg
- Oxidized glutathione: 0.245 g/kg
- pH to 9.5 ± 0.05 with 10N NaOH/glacial Acetic acid
Use buffer <7 hours.
Dilute into Refold buffer
(Refold solution will be
500 kg total in 500 L refold vessel)
Refolding parameters:
- Open to air (passive oxidation)
- Temperature <15°C
- pH = 9.5 ± 0.05
- Agitation = 200 rpm
TGF-β3 in refold buffer
Refold termination criteria:
- Maximum reaction time is 48 hours ± 30 minutes
- Stop reaction when 2 conditions occur:
- Redox reversal
- no further increase in CEX-HPLC dimer peak (<10% between any 3 samples)
Monitor refolding reaction progress
via redox and CEX-HPLC
(HPLC samples taken at T=0 and then again after first 12 hours and subsequently every 4 hours)
Refold Samples at each timepoint:
15mL QC Client retain
15mL CBSB retain
1mL CEX-HPLC
2 mL Western blot
2 mL Non-red SDS-PAGE
Continuous monitoring of redox potential, pH, agitation, temperature
Refolded TGF-β3 in refold buffer

FIG 19

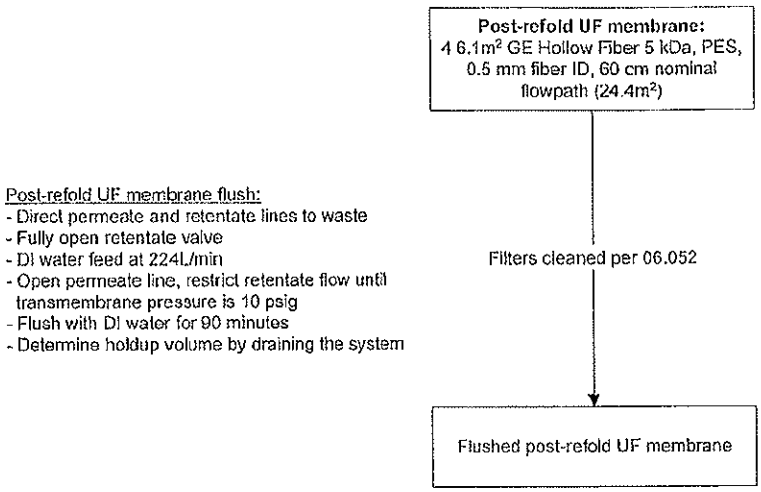

Post-refold UF membrane:
4 6.1m² GE Hollow Fiber 5 kDa, PES, 0.5 mm fiber ID, 60 cm nominal flowpath (24.4m²)
Post-refold UF membrane flush:
- Direct permeate and retentate lines to waste
- Fully open retentate valve
- DI water feed at 224L/min
- Open permeate line, restrict retentate flow until transmembrane pressure is 10 psig
- Flush with DI water for 90 minutes
- Determine holdup volume by draining the system
Filters cleaned per 06.052
Flushed post-refold UF membrane

FIG 20A

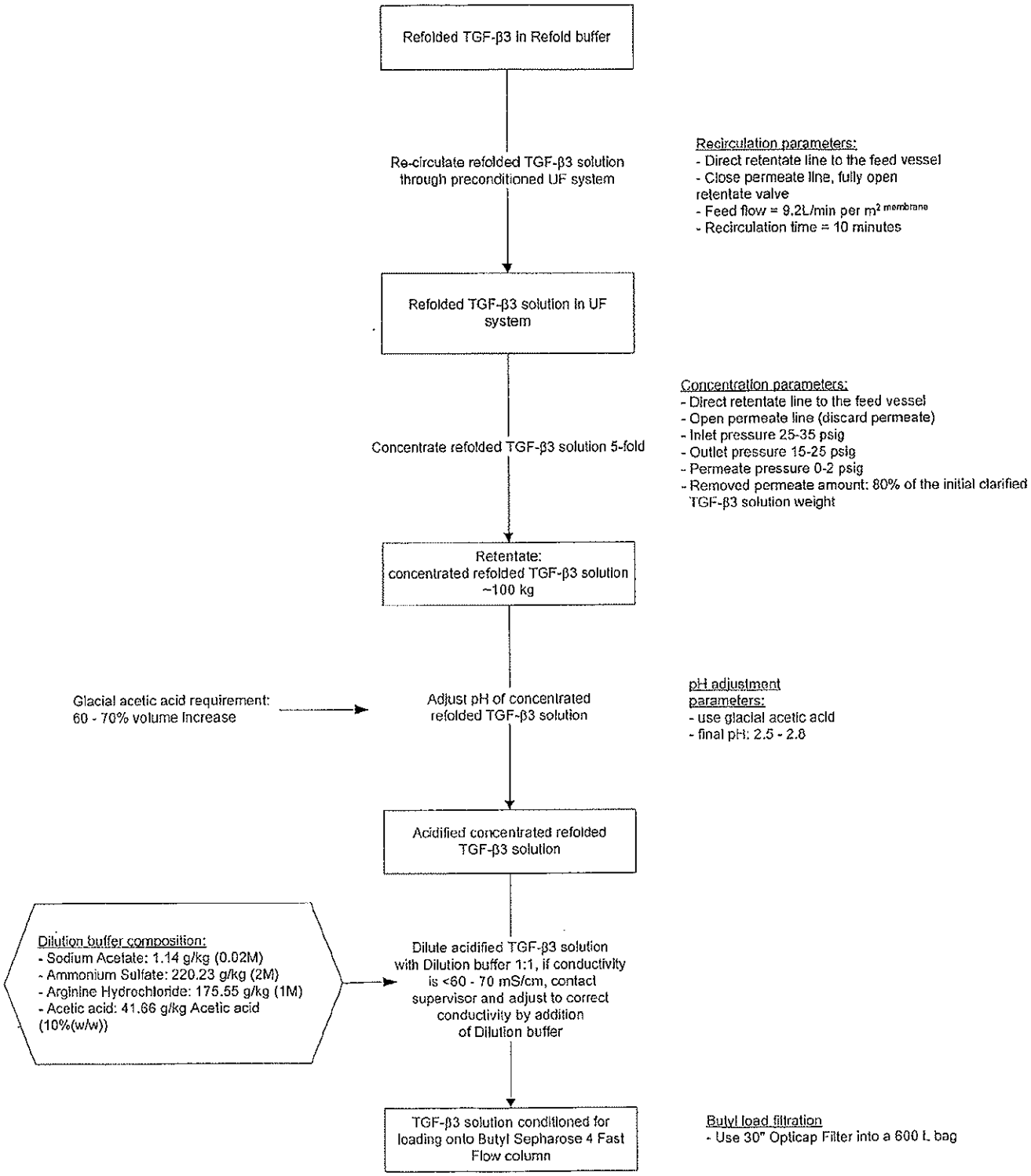

Refolded TGF-β3 in Refold buffer
Re-circulate refolded TGF-β3 solution through preconditioned UF system
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Feed flow = 9.2L/min per m² membrane
- Recirculation time = 10 minutes
Refolded TGF-β3 solution in UF system
Concentrate refolded TGF-β3 solution 5-fold
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Inlet pressure 25-35 psig
- Outlet pressure 15-25 psig
- Permeate pressure 0-2 psig
- Removed permeate amount: 80% of the initial clarified TGF-β3 solution weight
Retentate: concentrated refolded TGF-β3 solution ~100 kg
Glacial acetic acid requirement: 60 - 70% volume increase
Adjust pH of concentrated refolded TGF-β3 solution
pH adjustment parameters:
- use glacial acetic acid
- final pH: 2.5 - 2.8
Acidified concentrated refolded TGF-β3 solution
Dilution buffer composition:
- Sodium Acetate: 1.14 g/kg (0.02M)
- Ammonium Sulfate: 220.23 g/kg (2M)
- Arginine Hydrochloride: 175.55 g/kg (1M)
- Acetic acid: 41.66 g/kg Acetic acid (10%(w/w))
Dilute acidified TGF-β3 solution with Dilution buffer 1:1, if conductivity is <60 - 70 mS/cm, contact supervisor and adjust to correct conductivity by addition of Dilution buffer
TGF-β3 solution conditioned for loading onto Butyl Sepharose 4 Fast Flow column
Butyl load filtration
- Use 30" Opticap Filter into a 600 L bag

FIG 20B

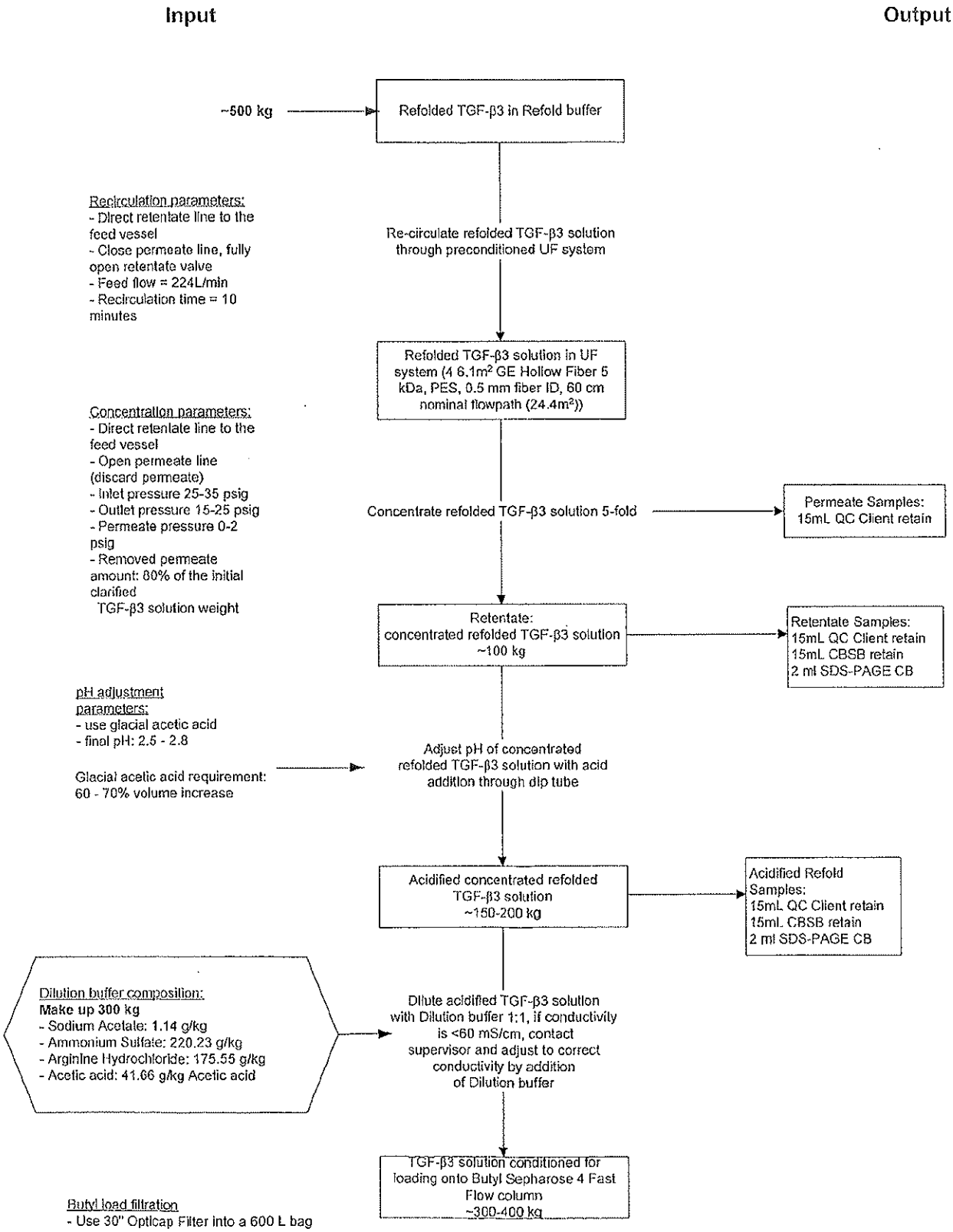

Input
Output
~500 kg
Refolded TGF-β3 in Refold buffer
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Feed flow = 224L/min
- Recirculation time = 10 minutes
Re-circulate refolded TGF-β3 solution through preconditioned UF system
Refolded TGF-β3 solution in UF system (4 6.1m² GE Hollow Fiber 5 kDa, PES, 0.5 mm fiber ID, 60 cm nominal flowpath (24.4m²))
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Inlet pressure 25-35 psig
- Outlet pressure 15-25 psig
- Permeate pressure 0-2 psig
- Removed permeate amount: 80% of the initial clarified TGF-β3 solution weight
Concentrate refolded TGF-β3 solution 5-fold
Permeate Samples: 15mL QC Client retain
Retentate: concentrated refolded TGF-β3 solution ~100 kg
Retentate Samples: 15mL QC Client retain 15mL CBSB retain 2 ml SDS-PAGE CB
pH adjustment parameters:
- use glacial acetic acid
- final pH: 2.5 - 2.8
Glacial acetic acid requirement: 60 - 70% volume increase
Adjust pH of concentrated refolded TGF-β3 solution with acid addition through dip tube
Acidified concentrated refolded TGF-β3 solution ~150-200 kg
Acidified Refold Samples: 15mL QC Client retain 15mL CBSB retain 2 ml SDS-PAGE CB
Dilution buffer composition:
Make up 300 kg
- Sodium Acetate: 1.14 g/kg
- Ammonium Sulfate: 220.23 g/kg
- Arginine Hydrochloride: 175.55 g/kg
- Acetic acid: 41.66 g/kg Acetic acid
Dilute acidified TGF-β3 solution with Dilution buffer 1:1, if conductivity is <60 mS/cm, contact supervisor and adjust to correct conductivity by addition of Dilution buffer
TGF-β3 solution conditioned for loading onto Butyl Sepharose 4 Fast Flow column ~300-400 kg
Butyl load filtration
- Use 30" Opticap Filter into a 600 L bag

FIG 21A

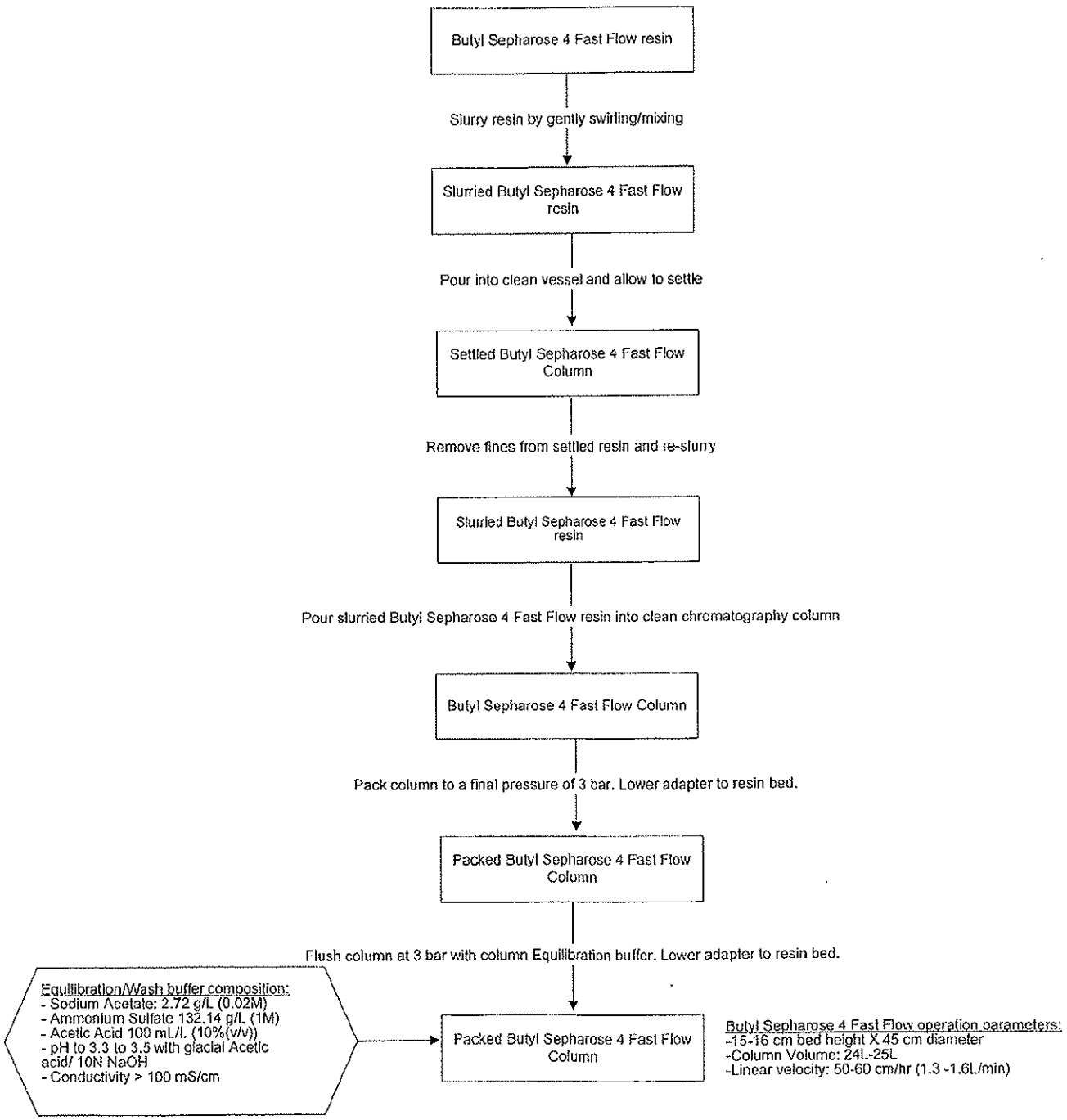

Butyl Sepharose 4 Fast Flow resin
Slurry resin by gently swirling/mixing
Slurried Butyl Sepharose 4 Fast Flow resin
Pour into clean vessel and allow to settle
Settled Butyl Sepharose 4 Fast Flow Column
Remove fines from settled resin and re-slurry
Slurried Butyl Sepharose 4 Fast Flow resin
Pour slurried Butyl Sepharose 4 Fast Flow resin into clean chromatography column
Butyl Sepharose 4 Fast Flow Column
Pack column to a final pressure of 3 bar. Lower adapter to resin bed.
Packed Butyl Sepharose 4 Fast Flow Column
Flush column at 3 bar with column Equilibration buffer. Lower adapter to resin bed.
Equilibration/Wash buffer composition:
- Sodium Acetate: 2.72 g/L (0.02M)
- Ammonium Sulfate 132.14 g/L (1M)
- Acetic Acid 100 mL/L (10%(v/v))
- pH to 3.3 to 3.5 with glacial Acetic acid/ 10N NaOH
- Conductivity > 100 mS/cm
Packed Butyl Sepharose 4 Fast Flow Column
Butyl Sepharose 4 Fast Flow operation parameters:
-15-16 cm bed height X 45 cm diameter
-Column Volume: 24L-25L
-Linear velocity: 50-60 cm/hr (1.3 -1.6L/min)

FIG 21B

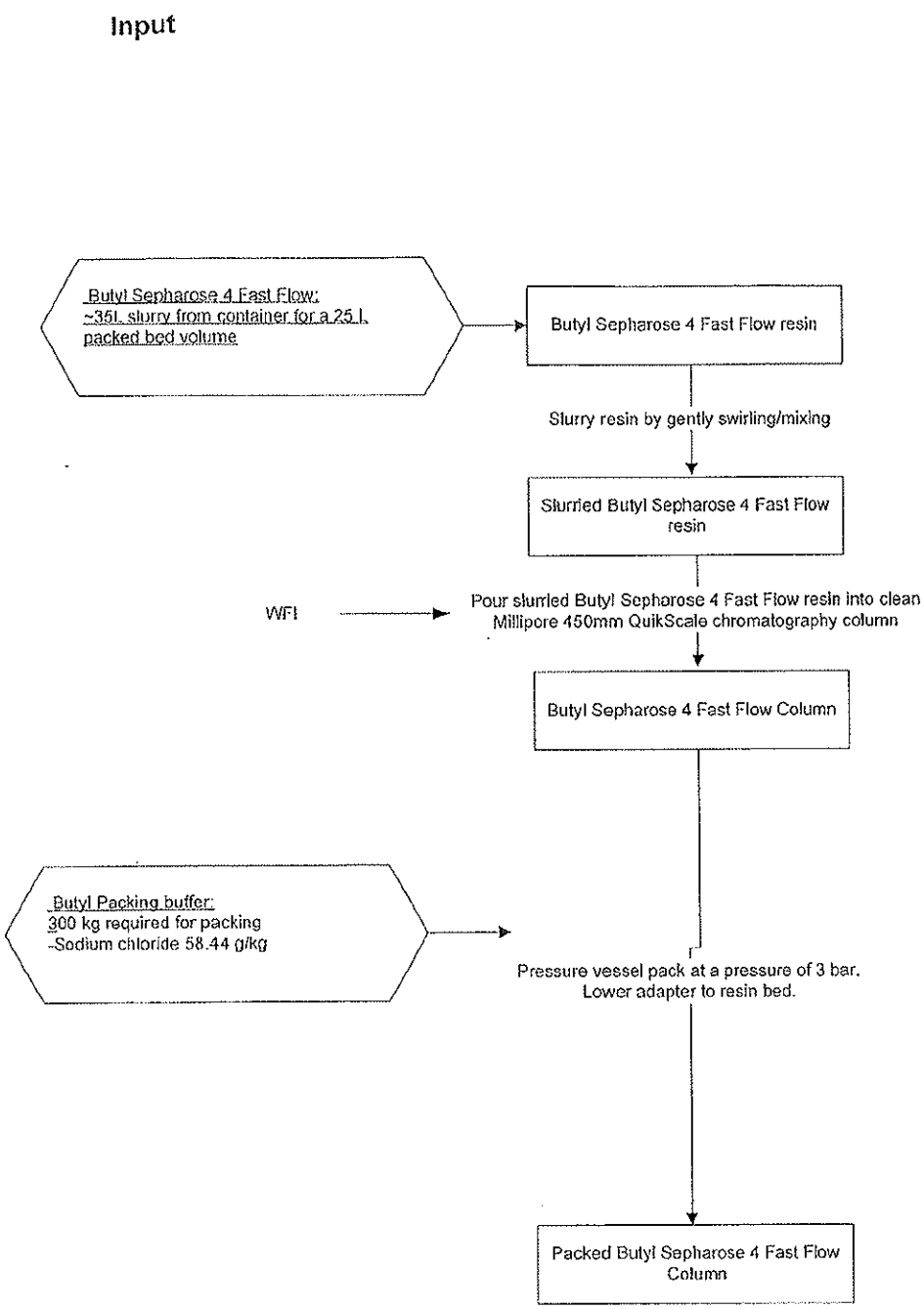

Input
Output
Butyl Sepharose 4 Fast Flow:
~35L slurry from container for a 25 L packed bed volume
Butyl Sepharose 4 Fast Flow resin
Slurry resin by gently swirling/mixing
Slurried Butyl Sepharose 4 Fast Flow resin
WFI
Pour slurried Butyl Sepharose 4 Fast Flow resin into clean Millipore 450mm QuikScale chromatography column
Butyl Sepharose 4 Fast Flow Column
Butyl Packing buffer:
300 kg required for packing
-Sodium chloride 58.44 g/kg
Pressure vessel pack at a pressure of 3 bar.
Lower adapter to resin bed.
Packed Butyl Sepharose 4 Fast Flow Column

FIG 21B continued

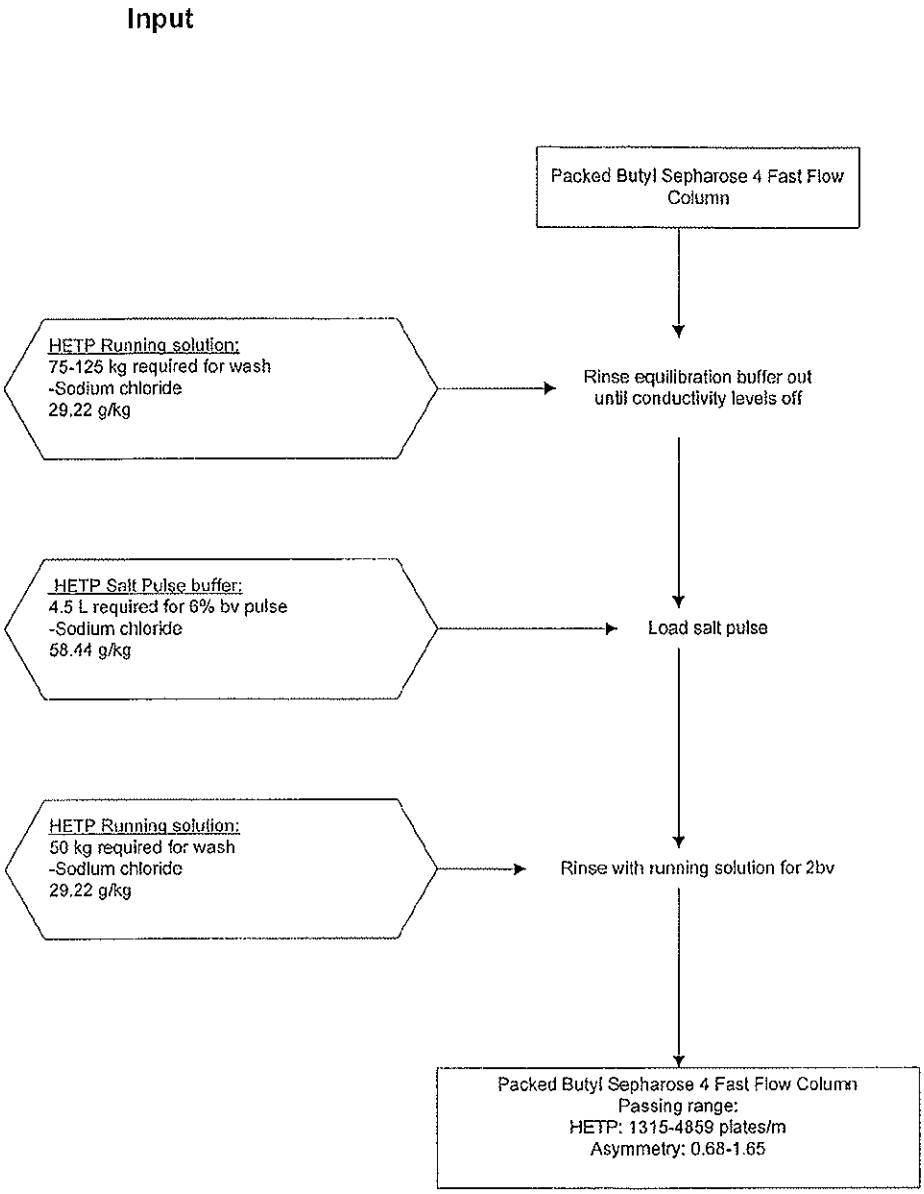


FIG 22A

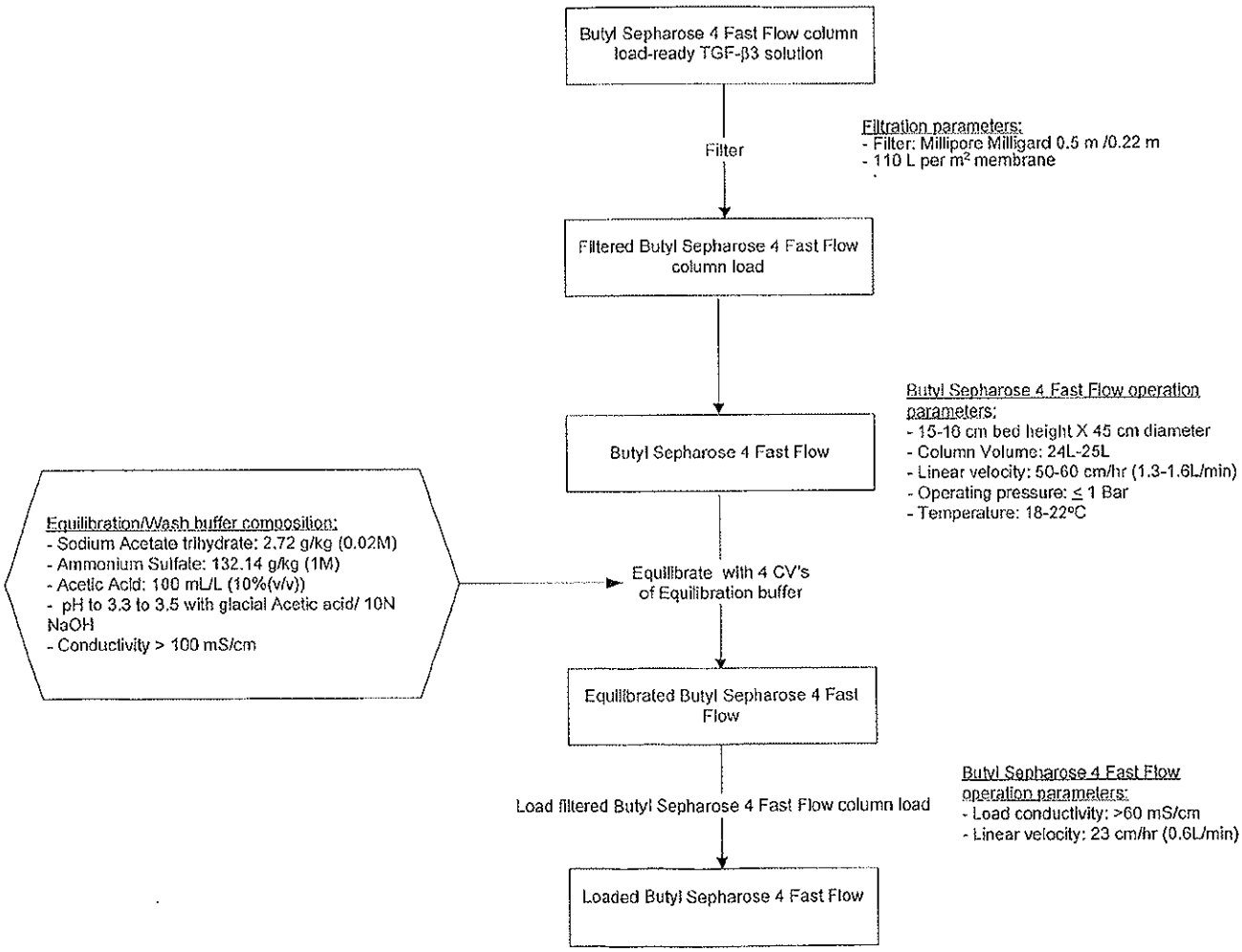

Butyl Sepharose 4 Fast Flow column load-ready TGF-β3 solution
Filter
Filtration parameters:
- Filter: Millipore Milligard 0.5 m /0.22 m
- 110 L per m² membrane
Filtered Butyl Sepharose 4 Fast Flow column load
Butyl Sepharose 4 Fast Flow
Butyl Sepharose 4 Fast Flow operation parameters:
- 15-16 cm bed height X 45 cm diameter
- Column Volume: 24L-25L
- Linear velocity: 50-60 cm/hr (1.3-1.6L/min)
- Operating pressure: ≤ 1 Bar
- Temperature: 18-22°C
Equilibration/Wash buffer composition:
- Sodium Acetate trihydrate: 2.72 g/kg (0.02M)
- Ammonium Sulfate: 132.14 g/kg (1M)
- Acetic Acid: 100 mL/L (10%(v/v))
- pH to 3.3 to 3.5 with glacial Acetic acid/ 10N NaOH
- Conductivity > 100 mS/cm
Equilibrate with 4 CV's of Equilibration buffer
Equilibrated Butyl Sepharose 4 Fast Flow
Load filtered Butyl Sepharose 4 Fast Flow column load
Butyl Sepharose 4 Fast Flow operation parameters:
- Load conductivity: >60 mS/cm
- Linear velocity: 23 cm/hr (0.6L/min)
Loaded Butyl Sepharose 4 Fast Flow

FIG 22A continued

Butyl Sepharose 4 Fast Flow (cont)

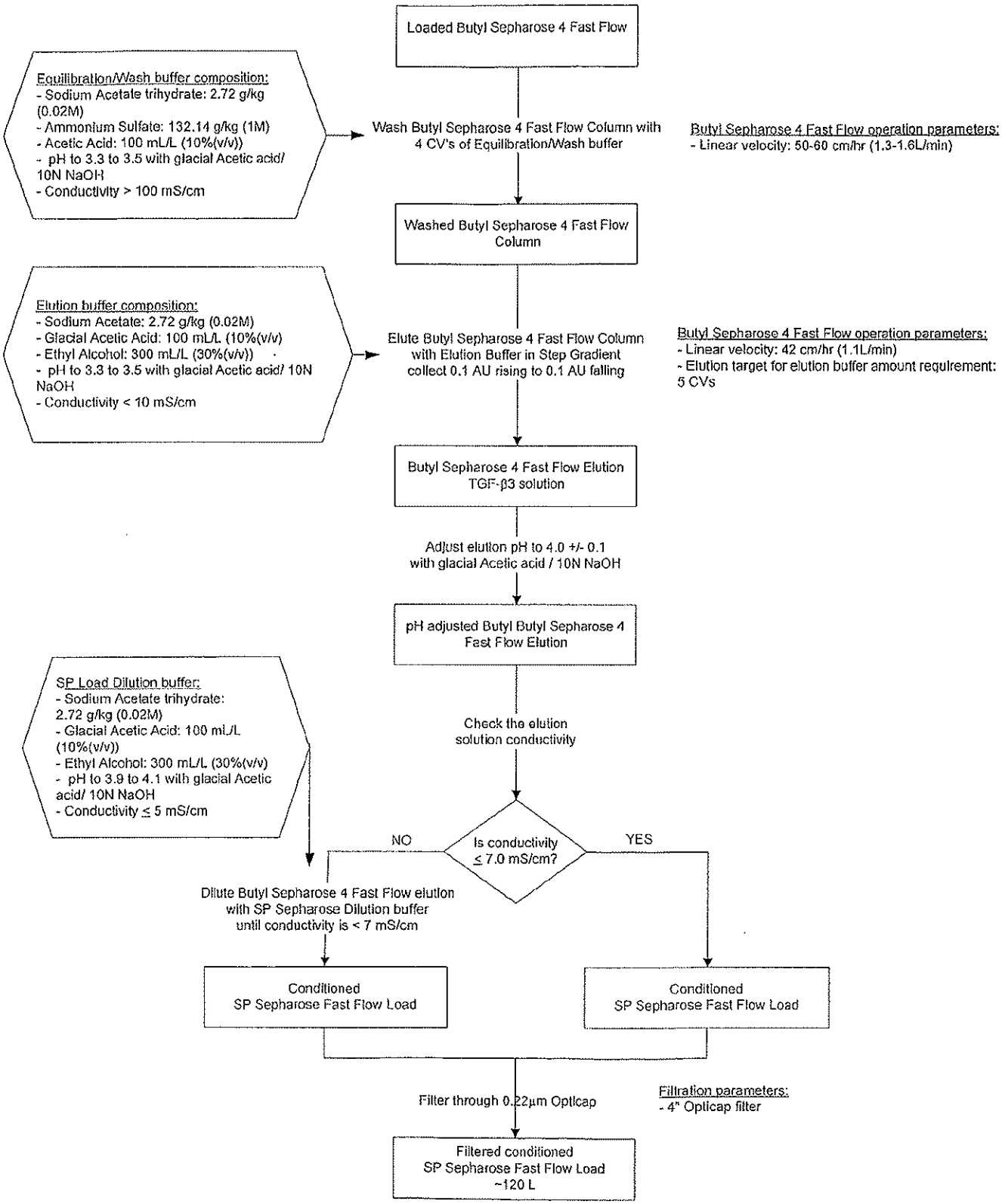


FIG 22B

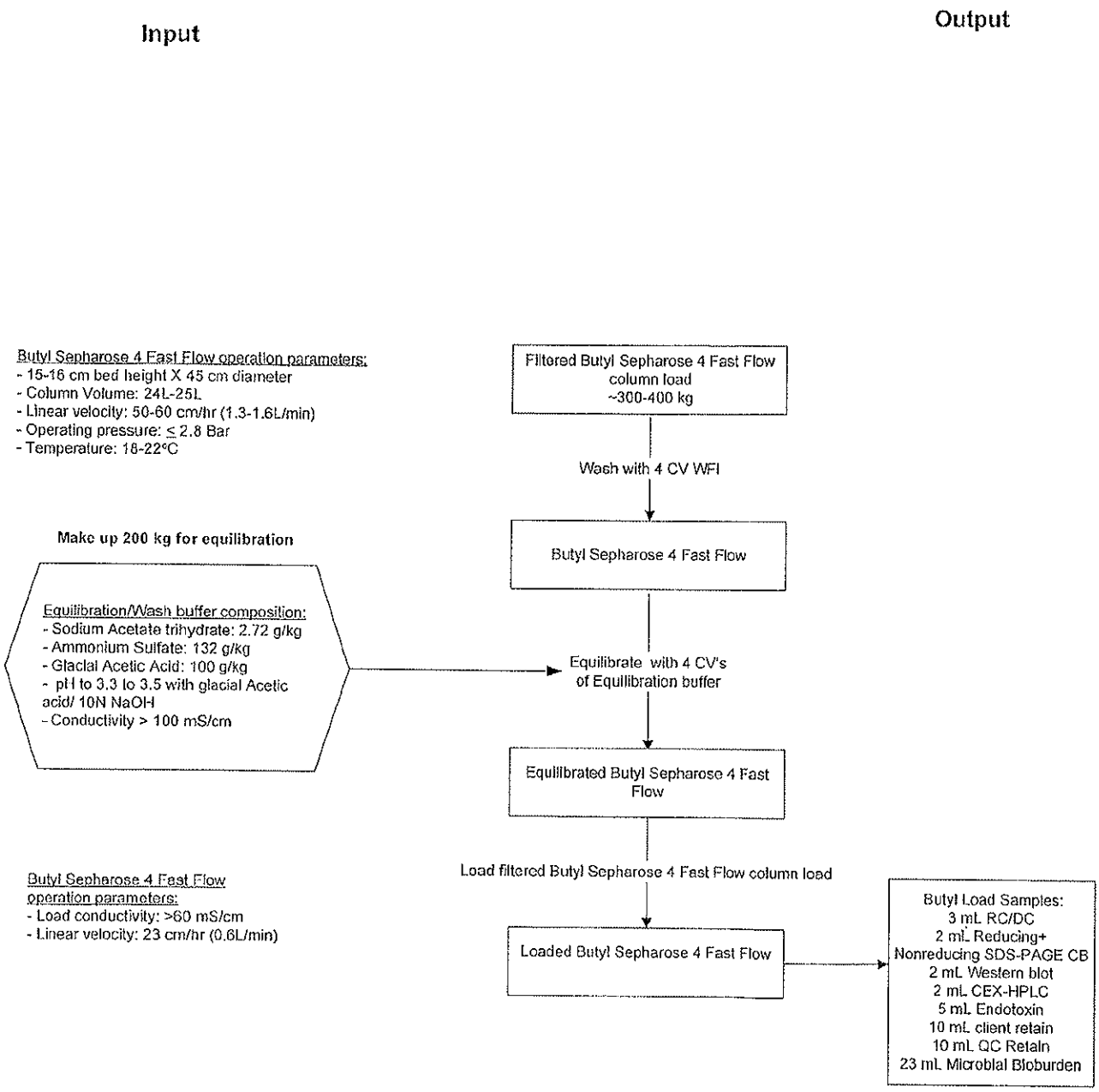

Input
Output
Butyl Sepharose 4 Fast Flow operation parameters:
- 15-16 cm bed height X 45 cm diameter
- Column Volume: 24L-25L
- Linear velocity: 50-60 cm/hr (1.3-1.6L/min)
- Operating pressure: ≤ 2.8 Bar
- Temperature: 18-22°C
Filtered Butyl Sepharose 4 Fast Flow column load ~300-400 kg
Wash with 4 CV WFI
Butyl Sepharose 4 Fast Flow
Make up 200 kg for equilibration
Equilibration/Wash buffer composition:
- Sodium Acetate trihydrate: 2.72 g/kg
- Ammonium Sulfate: 132 g/kg
- Glacial Acetic Acid: 100 g/kg
- pH to 3.3 to 3.5 with glacial Acetic acid/ 10N NaOH
- Conductivity > 100 mS/cm
Equilibrate with 4 CV's of Equilibration buffer
Equilibrated Butyl Sepharose 4 Fast Flow
Load filtered Butyl Sepharose 4 Fast Flow column load
Butyl Sepharose 4 Fast Flow operation parameters:
- Load conductivity: >60 mS/cm
- Linear velocity: 23 cm/hr (0.6L/min)
Loaded Butyl Sepharose 4 Fast Flow
Butyl Load Samples:
3 mL RC/DC
2 mL Reducing+ Nonreducing SDS-PAGE CB
2 mL Western blot
2 mL CEX-HPLC
5 mL Endotoxin
10 mL client retain
10 mL QC Retain
23 mL Microbial Bioburden

FIG 22B continued

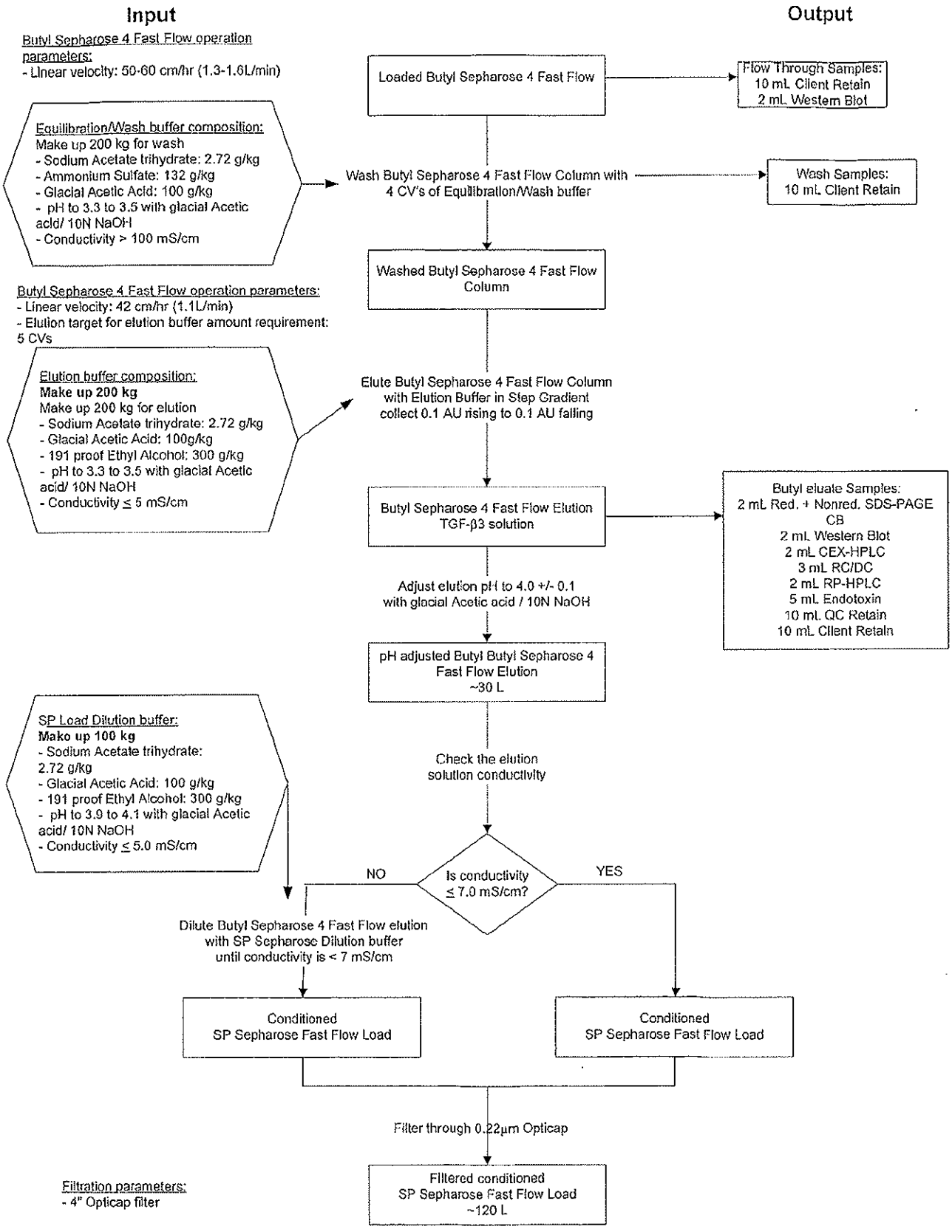
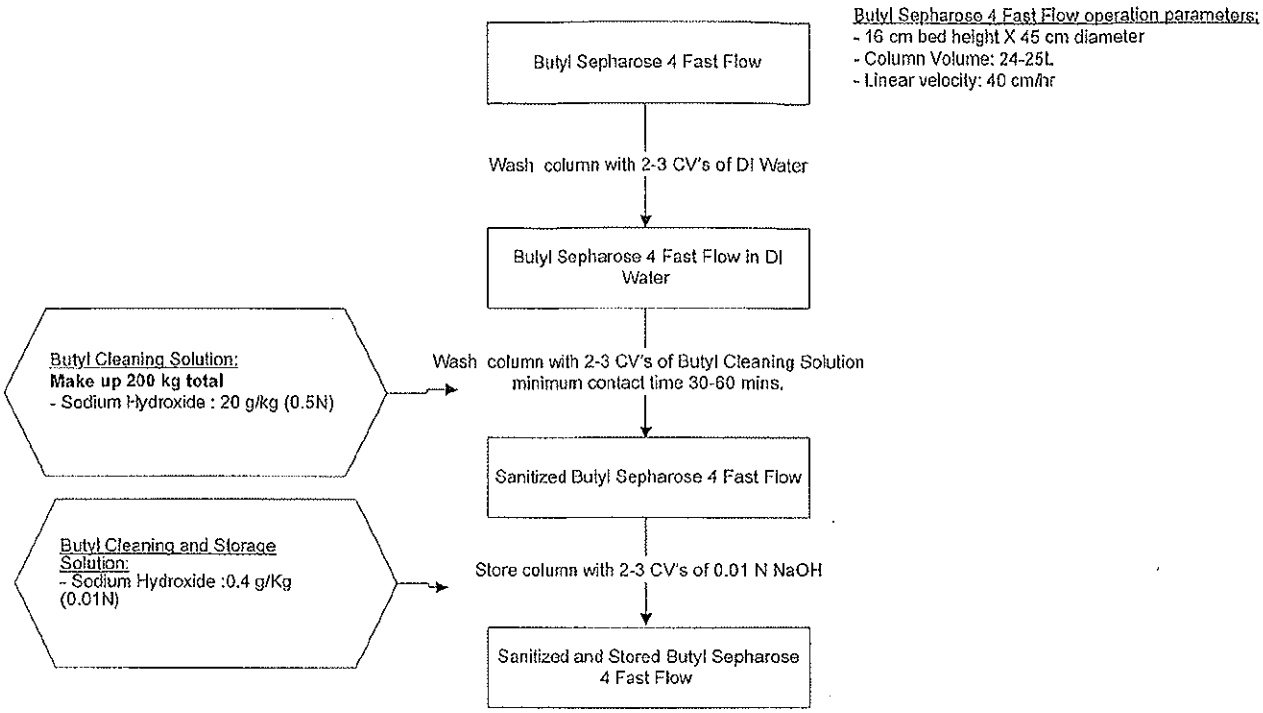


FIG 23A

Butyl Sepharose 4 Fast Flow Cleaning Post-Use



Butyl Sepharose 4 Fast Flow Cleaning Pre-Use/ Regeneration

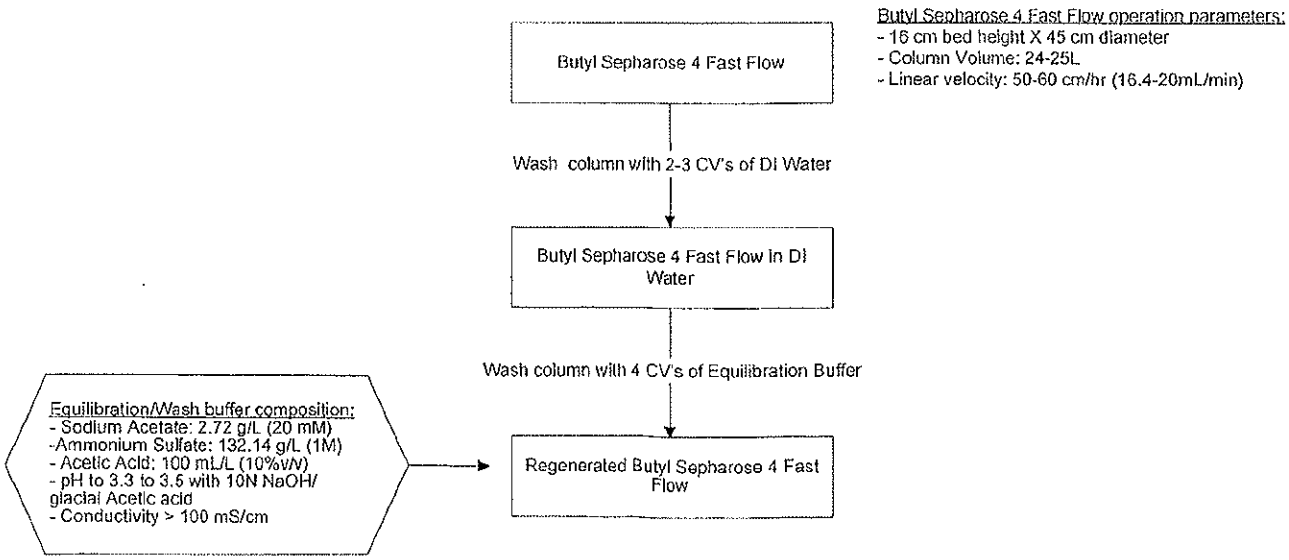


FIG 23B

Butyl Sepharose 4 Fast Flow Cleaning Pre-Use

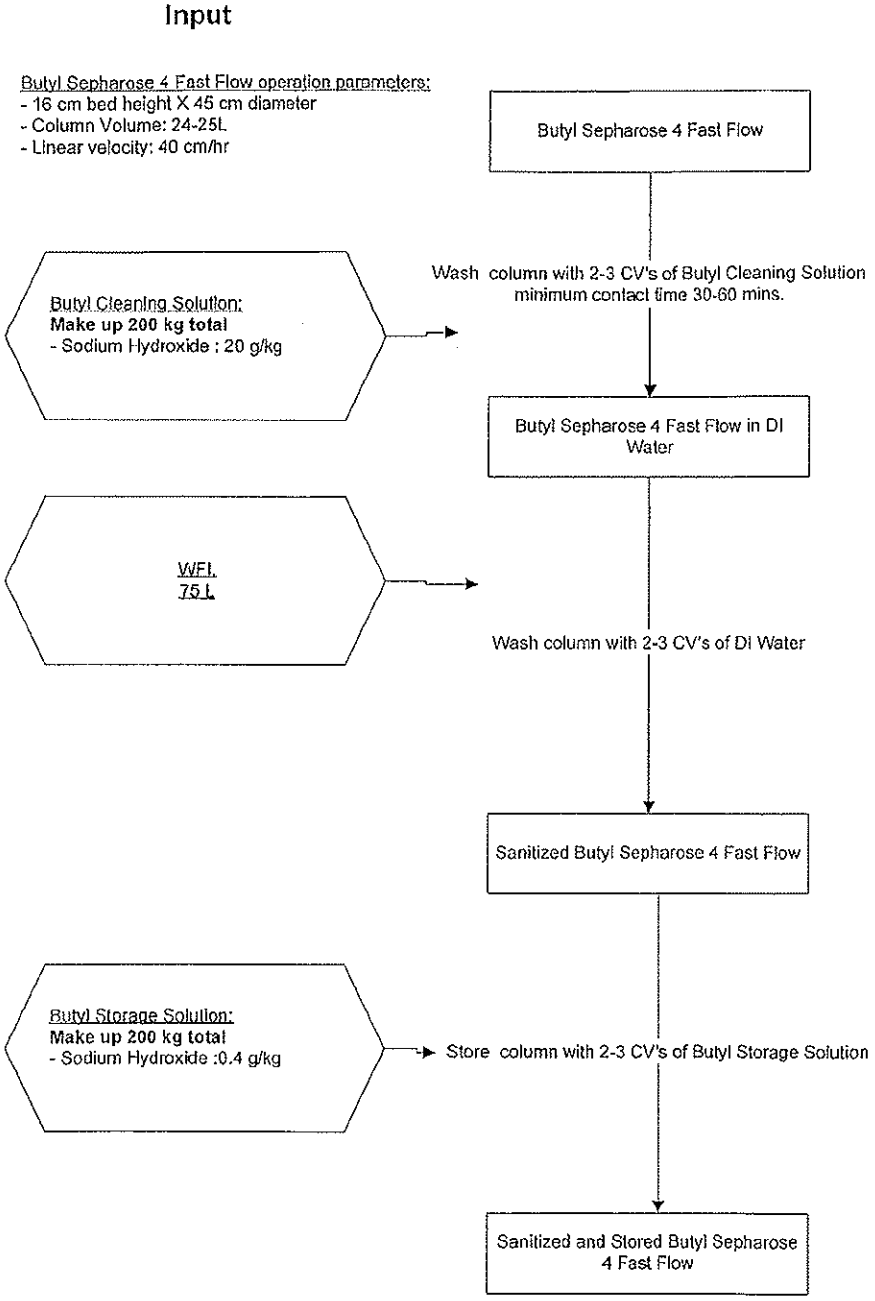


FIG 24

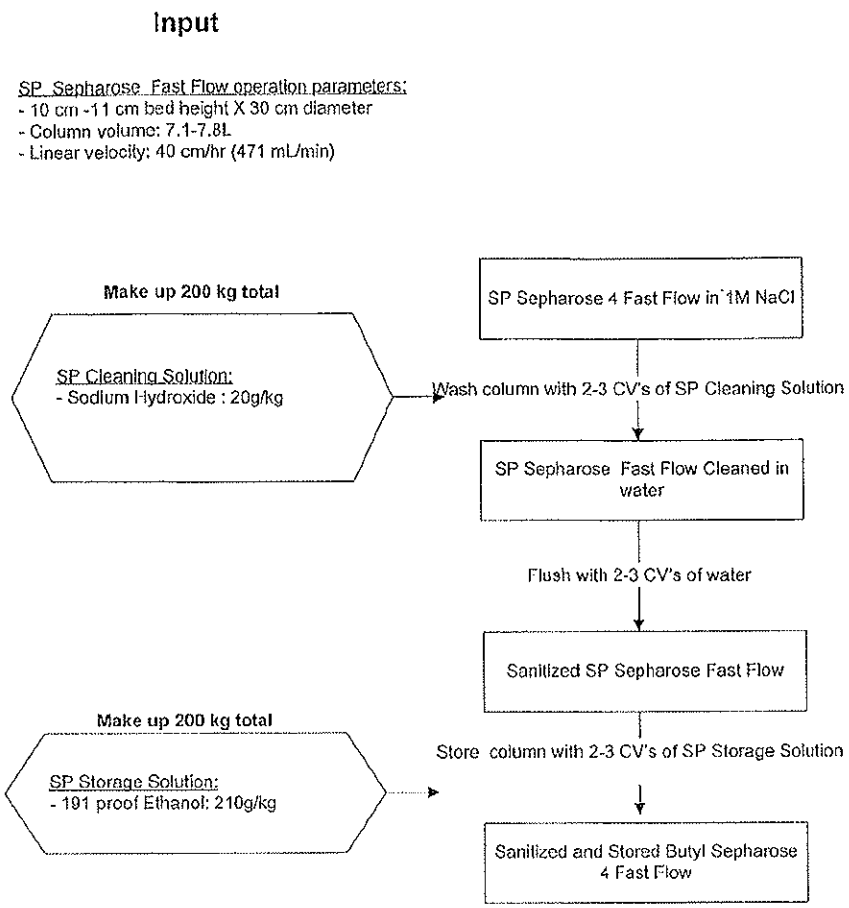

Input
Output
SP Sepharose Fast Flow operation parameters:
- 10 cm -11 cm bed height X 30 cm diameter
- Column volume: 7.1-7.8L
- Linear velocity: 40 cm/hr (471 mL/min)
Make up 200 kg total
SP Cleaning Solution:
- Sodium Hydroxide : 20g/kg
SP Sepharose 4 Fast Flow in 1M NaCl
Wash column with 2-3 CV's of SP Cleaning Solution
SP Sepharose Fast Flow Cleaned in water
Flush with 2-3 CV's of water
Sanitized SP Sepharose Fast Flow
Make up 200 kg total
SP Storage Solution:
- 191 proof Ethanol: 210g/kg
Store column with 2-3 CV's of SP Storage Solution
Sanitized and Stored Butyl Sepharose 4 Fast Flow

FIG 25

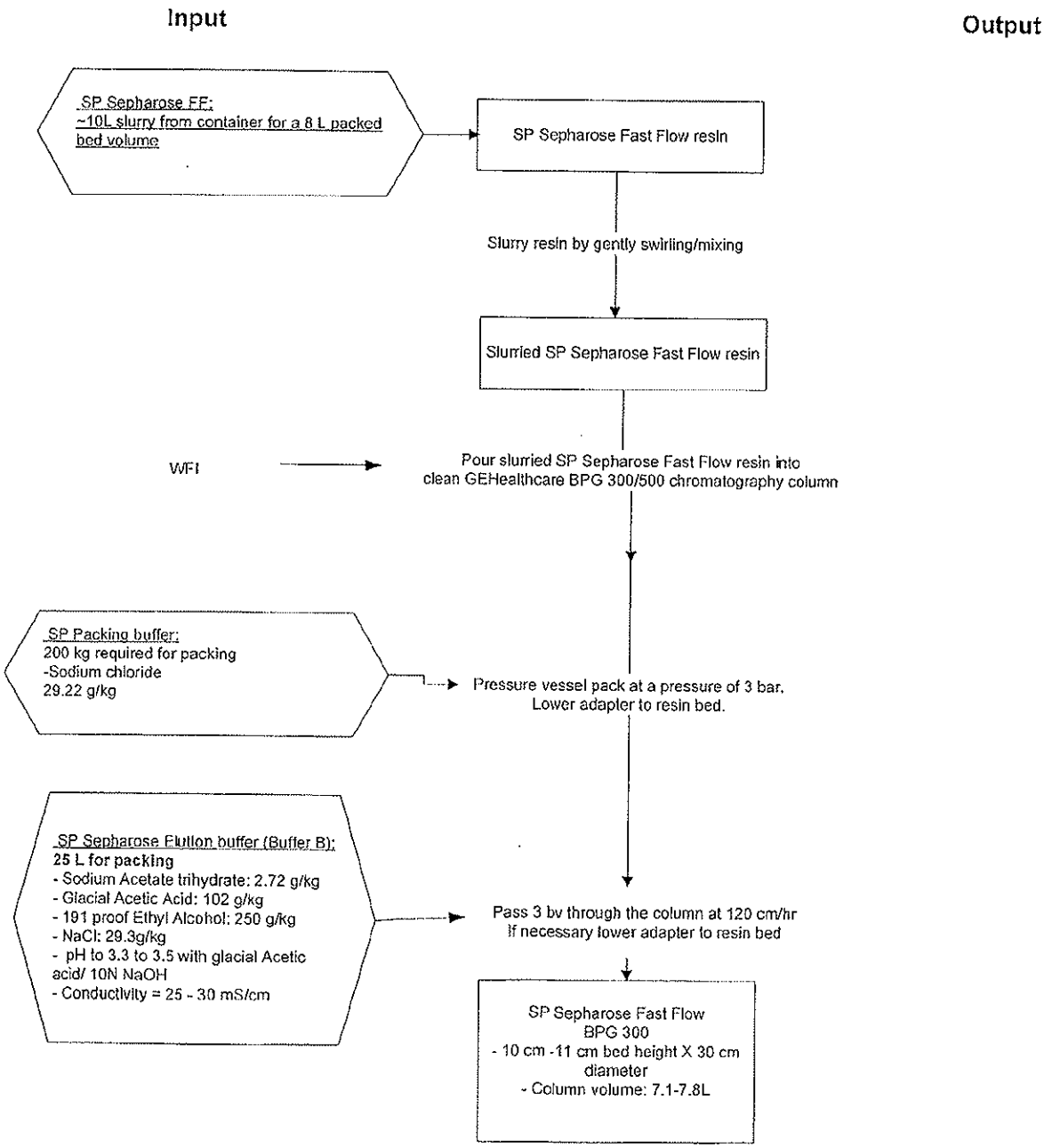

Input
Output
SP Sepharose FF:
~10L slurry from container for a 8 L packed bed volume
SP Sepharose Fast Flow resin
Slurry resin by gently swirling/mixing
Slurried SP Sepharose Fast Flow resin
WFI
Pour slurried SP Sepharose Fast Flow resin into clean GEHealthcare BPG 300/500 chromatography column
SP Packing buffer:
200 kg required for packing
-Sodium chloride
29.22 g/kg
Pressure vessel pack at a pressure of 3 bar.
Lower adapter to resin bed.
SP Sepharose Elution buffer (Buffer B):
25 L for packing
- Sodium Acetate trihydrate: 2.72 g/kg
- Glacial Acetic Acid: 102 g/kg
- 191 proof Ethyl Alcohol: 250 g/kg
- NaCl: 29.3g/kg
- pH to 3.3 to 3.5 with glacial Acetic acid/ 10N NaOH
- Conductivity = 25 - 30 mS/cm
Pass 3 bv through the column at 120 cm/hr
If necessary lower adapter to resin bed
SP Sepharose Fast Flow
BPG 300
- 10 cm -11 cm bed height X 30 cm diameter
- Column volume: 7.1-7.8L

FIG 25 continued

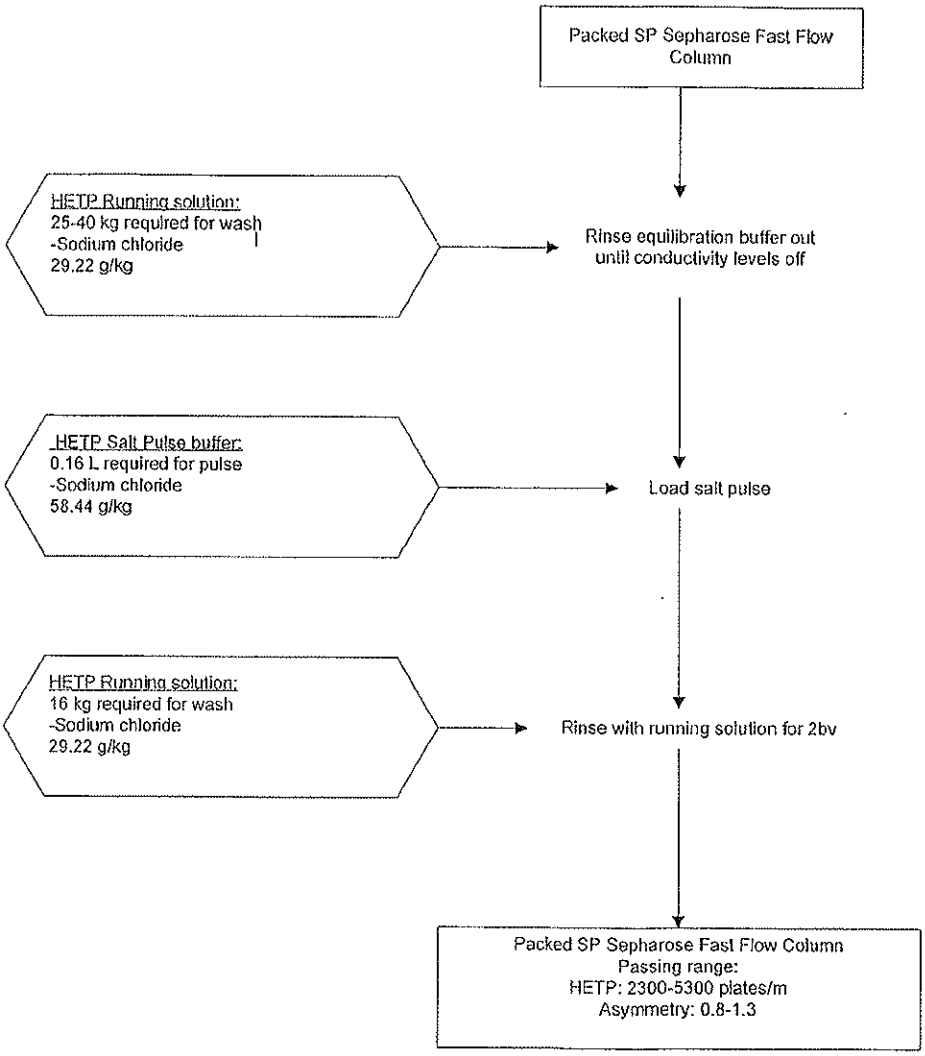


FIG 26A

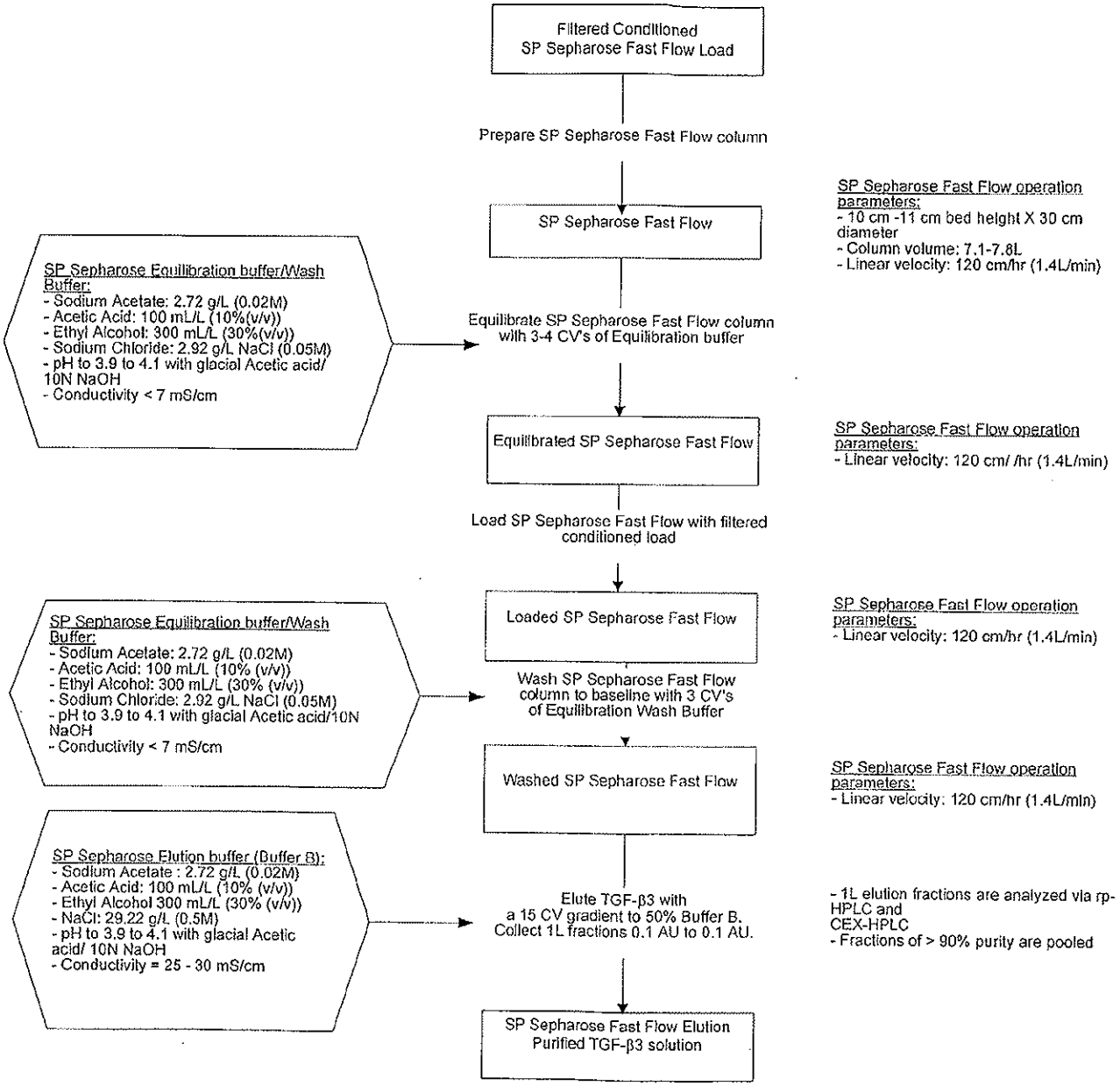

Filtered Conditioned SP Sepharose Fast Flow Load
Prepare SP Sepharose Fast Flow column
SP Sepharose Fast Flow
SP Sepharose Fast Flow operation parameters:
- 10 cm -11 cm bed height X 30 cm diameter
- Column volume: 7.1-7.8L
- Linear velocity: 120 cm/hr (1.4L/min)
SP Sepharose Equilibration buffer/Wash Buffer:
- Sodium Acetate: 2.72 g/L (0.02M)
- Acetic Acid: 100 mL/L (10%(v/v))
- Ethyl Alcohol: 300 mL/L (30%(v/v))
- Sodium Chloride: 2.92 g/L NaCl (0.05M)
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
- Conductivity < 7 mS/cm
Equilibrate SP Sepharose Fast Flow column with 3-4 CV's of Equilibration buffer
Equilibrated SP Sepharose Fast Flow
SP Sepharose Fast Flow operation parameters:
- Linear velocity: 120 cm/ /hr (1.4L/min)
Load SP Sepharose Fast Flow with filtered conditioned load
Loaded SP Sepharose Fast Flow
SP Sepharose Fast Flow operation parameters:
- Linear velocity: 120 cm/hr (1.4L/min)
SP Sepharose Equilibration buffer/Wash Buffer:
- Sodium Acetate: 2.72 g/L (0.02M)
- Acetic Acid: 100 mL/L (10% (v/v))
- Ethyl Alcohol: 300 mL/L (30% (v/v))
- Sodium Chloride: 2.92 g/L NaCl (0.05M)
- pH to 3.9 to 4.1 with glacial Acetic acid/10N NaOH
- Conductivity < 7 mS/cm
Wash SP Sepharose Fast Flow column to baseline with 3 CV's of Equilibration Wash Buffer
Washed SP Sepharose Fast Flow
SP Sepharose Fast Flow operation parameters:
- Linear velocity: 120 cm/hr (1.4L/min)
SP Sepharose Elution buffer (Buffer B):
- Sodium Acetate : 2.72 g/L (0.02M)
- Acetic Acid: 100 mL/L (10% (v/v))
- Ethyl Alcohol 300 mL/L (30% (v/v))
- NaCl: 29.22 g/L (0.5M)
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
- Conductivity = 25 - 30 mS/cm
Elute TGF-β3 with a 15 CV gradient to 50% Buffer B. Collect 1L fractions 0.1 AU to 0.1 AU.
- 1L elution fractions are analyzed via rp-HPLC and CEX-HPLC
- Fractions of > 90% purity are pooled
SP Sepharose Fast Flow Elution Purified TGF-β3 solution

FIG 26B

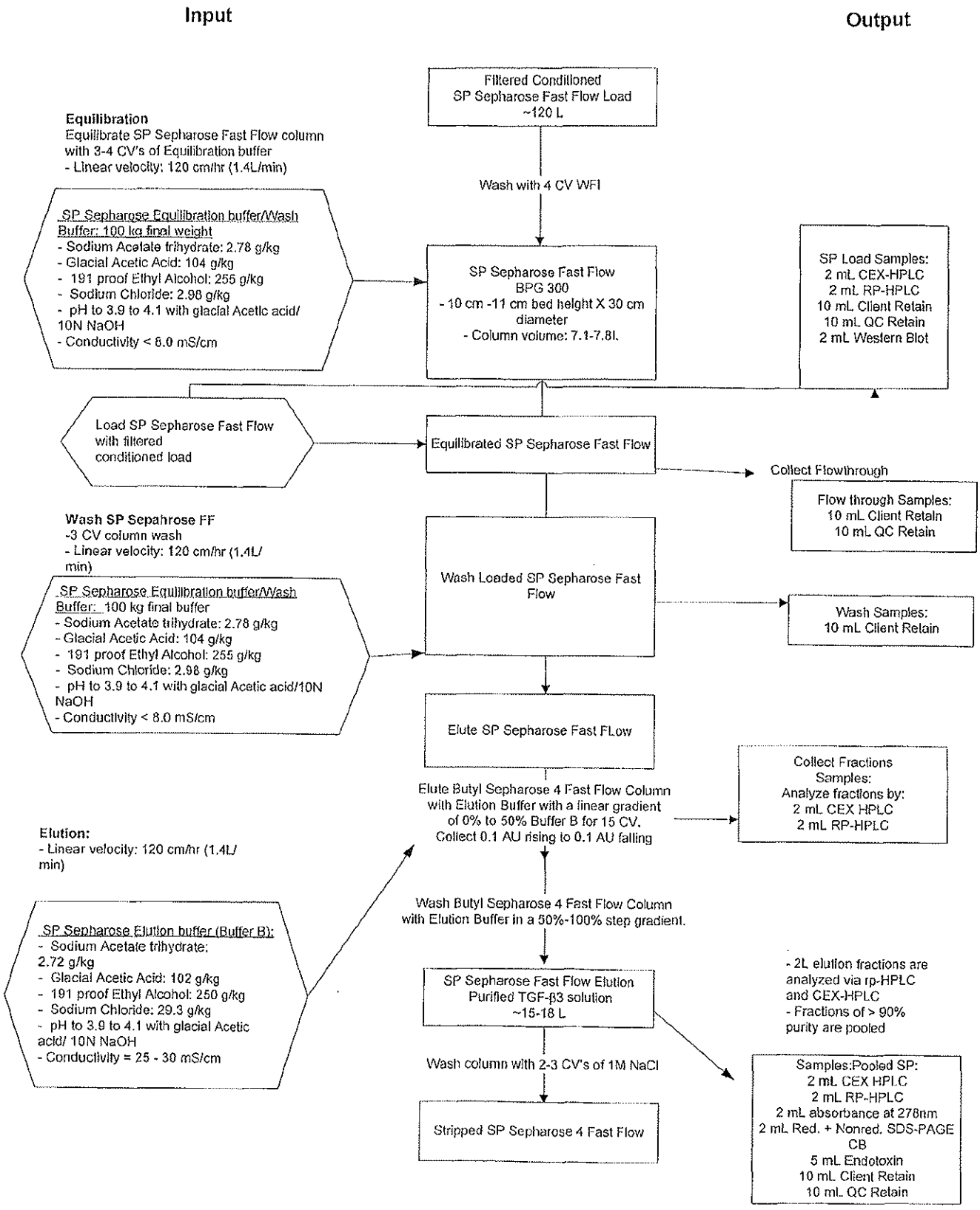
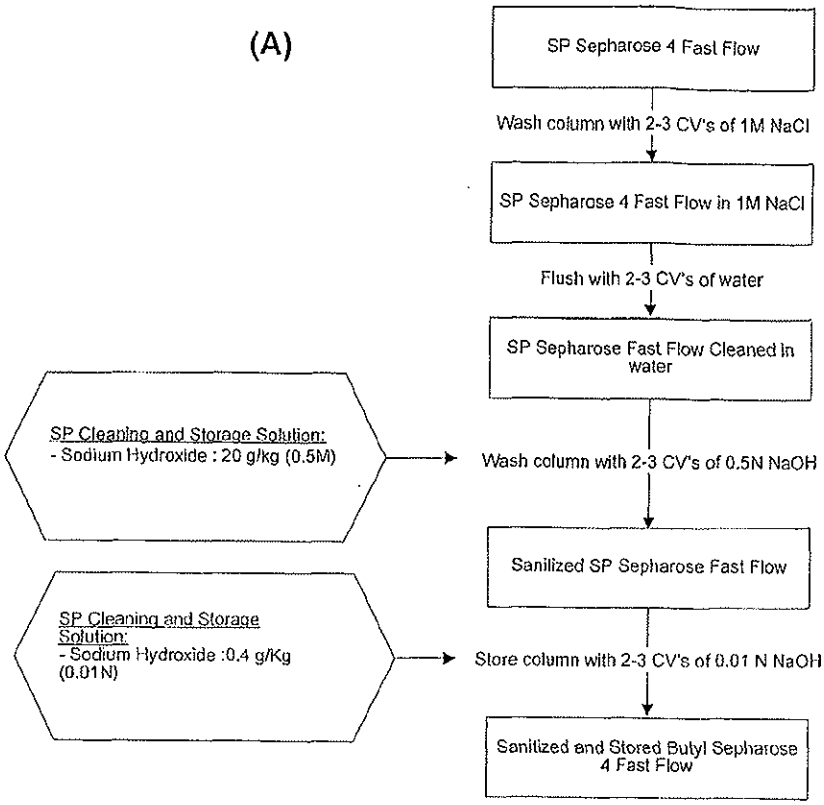

Input
Output
Filtered Conditioned SP Sepharose Fast Flow Load ~120 L
Equilibration
Equilibrate SP Sepharose Fast Flow column with 3-4 CV's of Equilibration buffer
- Linear velocity: 120 cm/hr (1.4L/min)
Wash with 4 CV WFI
SP Sepharose Equilibration buffer/Wash Buffer: 100 kg final weight
- Sodium Acetate trihydrate: 2.78 g/kg
- Glacial Acetic Acid: 104 g/kg
- 191 proof Ethyl Alcohol: 255 g/kg
- Sodium Chloride: 2.98 g/kg
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
- Conductivity < 8.0 mS/cm
SP Sepharose Fast Flow BPG 300
- 10 cm -11 cm bed height X 30 cm diameter
- Column volume: 7.1-7.8L
SP Load Samples:
2 mL CEX-HPLC
2 mL RP-HPLC
10 mL Client Retain
10 mL QC Retain
2 mL Western Blot
Load SP Sepharose Fast Flow with filtered conditioned load
Equilibrated SP Sepharose Fast Flow
Collect Flowthrough
Flow through Samples:
10 mL Client Retain
10 mL QC Retain
Wash SP Sepahrose FF
-3 CV column wash
- Linear velocity: 120 cm/hr (1.4L/ min)
SP Sepharose Equilibration buffer/Wash Buffer: 100 kg final buffer
- Sodium Acetate trihydrate: 2.78 g/kg
- Glacial Acetic Acid: 104 g/kg
- 191 proof Ethyl Alcohol: 255 g/kg
- Sodium Chloride: 2.98 g/kg
- pH to 3.9 to 4.1 with glacial Acetic acid/10N NaOH
- Conductivity < 8.0 mS/cm
Wash Loaded SP Sepharose Fast Flow
Wash Samples:
10 mL Client Retain
Elute SP Sepharose Fast FLow
Collect Fractions Samples:
Analyze fractions by:
2 mL CEX HPLC
2 mL RP-HPLC
Elute Butyl Sepharose 4 Fast Flow Column with Elution Buffer with a linear gradient of 0% to 50% Buffer B for 15 CV. Collect 0.1 AU rising to 0.1 AU falling
Elution:
- Linear velocity: 120 cm/hr (1.4L/ min)
Wash Butyl Sepharose 4 Fast Flow Column with Elution Buffer in a 50%-100% step gradient.
SP Sepharose Elution buffer (Buffer B):
- Sodium Acetate trihydrate: 2.72 g/kg
- Glacial Acetic Acid: 102 g/kg
- 191 proof Ethyl Alcohol: 250 g/kg
- Sodium Chloride: 29.3 g/kg
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
- Conductivity = 25 - 30 mS/cm
SP Sepharose Fast Flow Elution Purified TGF-β3 solution ~15-18 L
- 2L elution fractions are analyzed via rp-HPLC and CEX-HPLC
- Fractions of > 90% purity are pooled
Wash column with 2-3 CV's of 1M NaCl
Stripped SP Sepharose 4 Fast Flow
Samples:Pooled SP:
2 mL CEX HPLC
2 mL RP-HPLC
2 mL absorbance at 278nm
2 mL Red. + Nonred. SDS-PAGE CB
5 mL Endotoxin
10 mL Client Retain
10 mL QC Retain

FIG 27

(A)


SP Sepharose 4 Fast Flow
SP Sepharose Fast Flow operation parameters:
- 10 cm -11 cm bed height X 30 cm diameter
- Column volume: 7.1-7.8L
- Linear velocity: 40 cm/hr (471 mL/min)
Wash column with 2-3 CV's of 1M NaCl
SP Sepharose 4 Fast Flow in 1M NaCl
Flush with 2-3 CV's of water
SP Sepharose Fast Flow Cleaned in water
SP Cleaning and Storage Solution:
- Sodium Hydroxide : 20 g/kg (0.5M)
Wash column with 2-3 CV's of 0.5N NaOH
Sanilized SP Sepharose Fast Flow
SP Cleaning and Storage Solution:
- Sodium Hydroxide :0.4 g/Kg (0.01N)
Store column with 2-3 CV's of 0.01 N NaOH
Sanitized and Stored Butyl Sepharose 4 Fast Flow

(B)

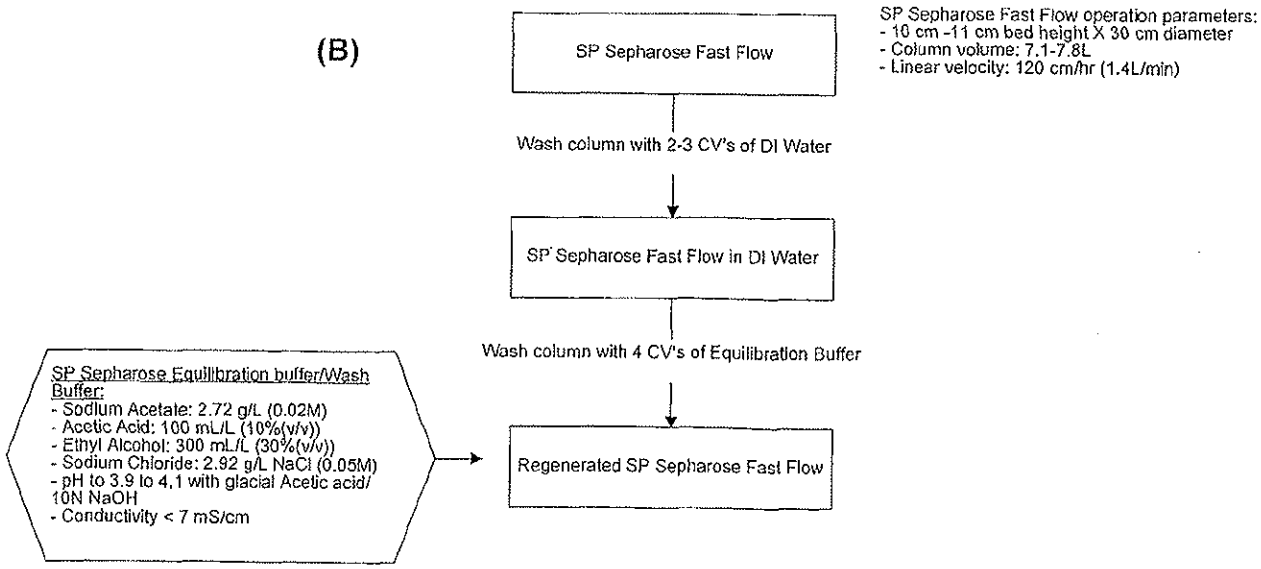

SP Sepharose Fast Flow
SP Sepharose Fast Flow operation parameters:
- 10 cm -11 cm bed height X 30 cm diameter
- Column volume: 7.1-7.8L
- Linear velocity: 120 cm/hr (1.4L/min)
Wash column with 2-3 CV's of DI Water
SP Sepharose Fast Flow in DI Water
Wash column with 4 CV's of Equilibration Buffer
SP Sepharose Equilibration buffer/Wash Buffer:
- Sodium Acetate: 2.72 g/L (0.02M)
- Acetic Acid: 100 mL/L (10%(v/v))
- Ethyl Alcohol: 300 mL/L (30%(v/v))
- Sodium Chloride: 2.92 g/L NaCl (0.05M)
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
- Conductivity < 7 mS/cm
Regenerated SP Sepharose Fast Flow

FIG 28A

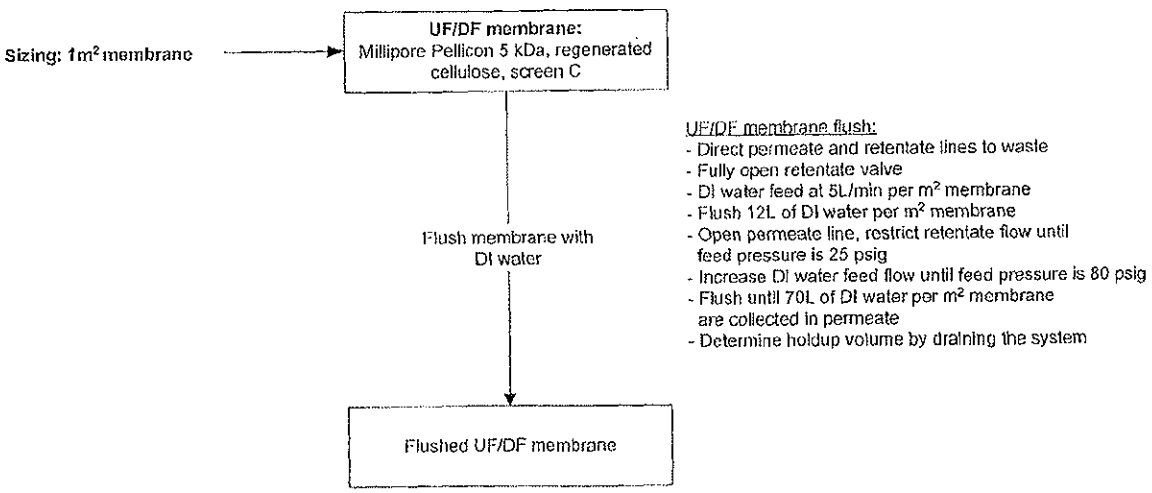

Sizing: 1m² membrane
UF/DF membrane:
Millipore Pellicon 5 kDa, regenerated cellulose, screen C
Flush membrane with DI water
Flushed UF/DF membrane
UF/DF membrane flush:
- Direct permeate and retentate lines to waste
- Fully open retentate valve
- DI water feed at 5L/min per m² membrane
- Flush 12L of DI water per m² membrane
- Open permeate line, restrict retentate flow until feed pressure is 25 psig
- Increase DI water feed flow until feed pressure is 80 psig
- Flush until 70L of DI water per m² membrane are collected in permeate
- Determine holdup volume by draining the system

FIG 28B

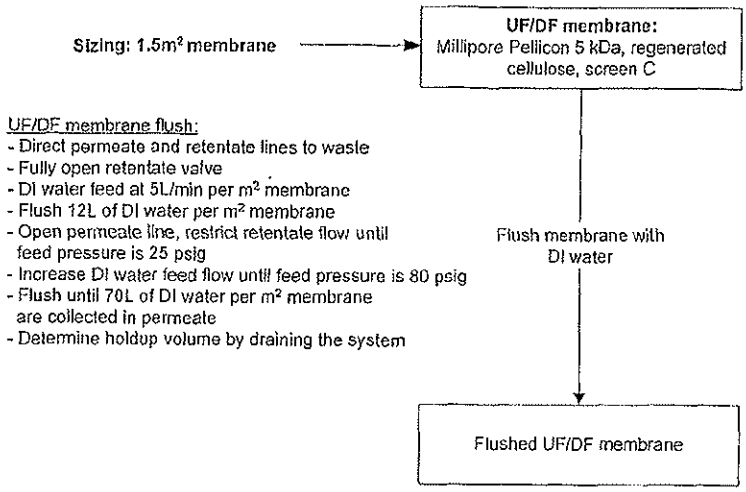

Sizing: 1.5m² membrane
UF/DF membrane:
Millipore Pellicon 5 kDa, regenerated cellulose, screen C
UF/DF membrane flush:
- Direct permeate and retentate lines to waste
- Fully open retentate valve
- DI water feed at 5L/min per m² membrane
- Flush 12L of DI water per m² membrane
- Open permeate line, restrict retentate flow until feed pressure is 25 psig
- Increase DI water feed flow until feed pressure is 80 psig
- Flush until 70L of DI water per m² membrane are collected in permeate
- Determine holdup volume by draining the system
Flush membrane with DI water
Flushed UF/DF membrane

FIG 29A

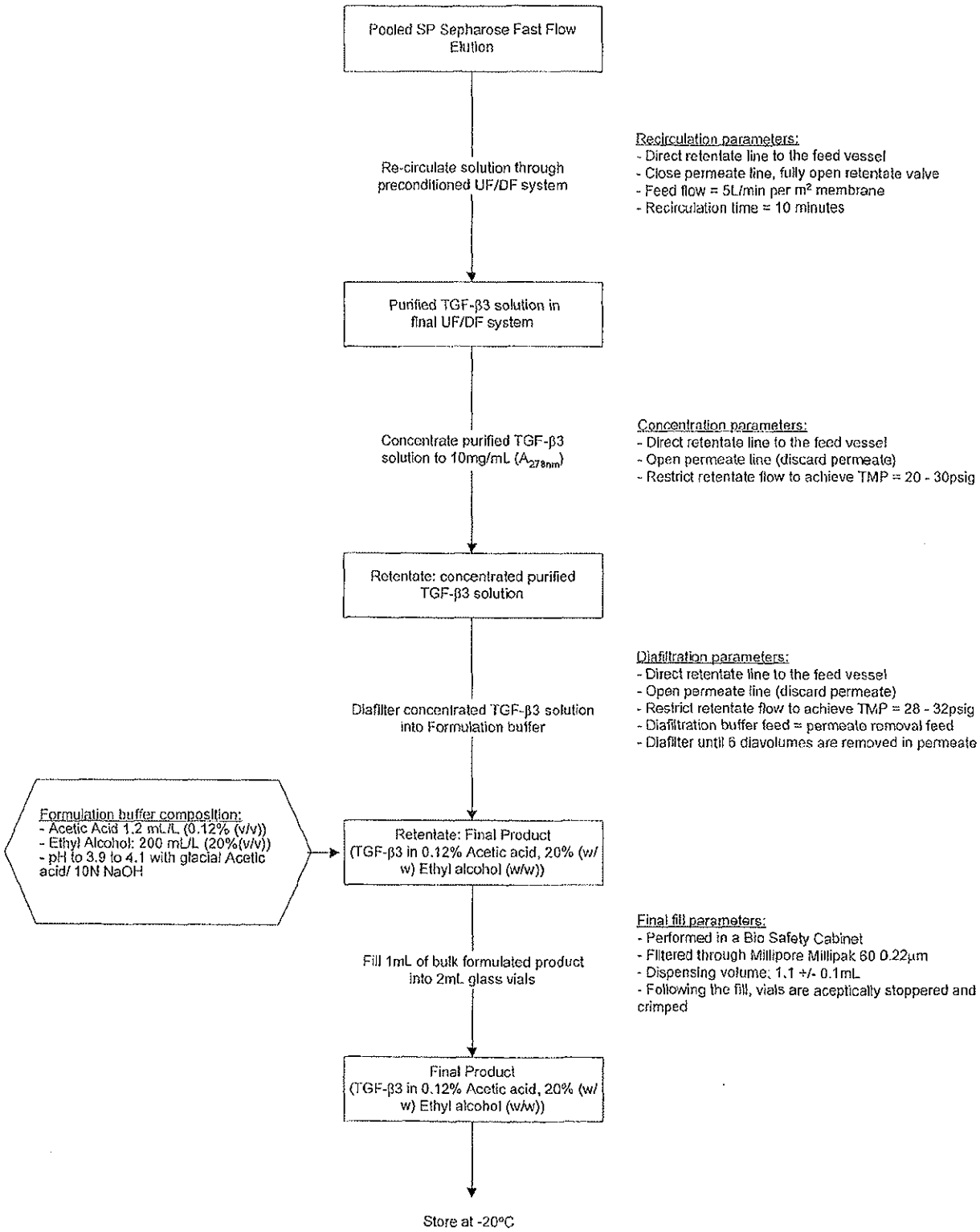
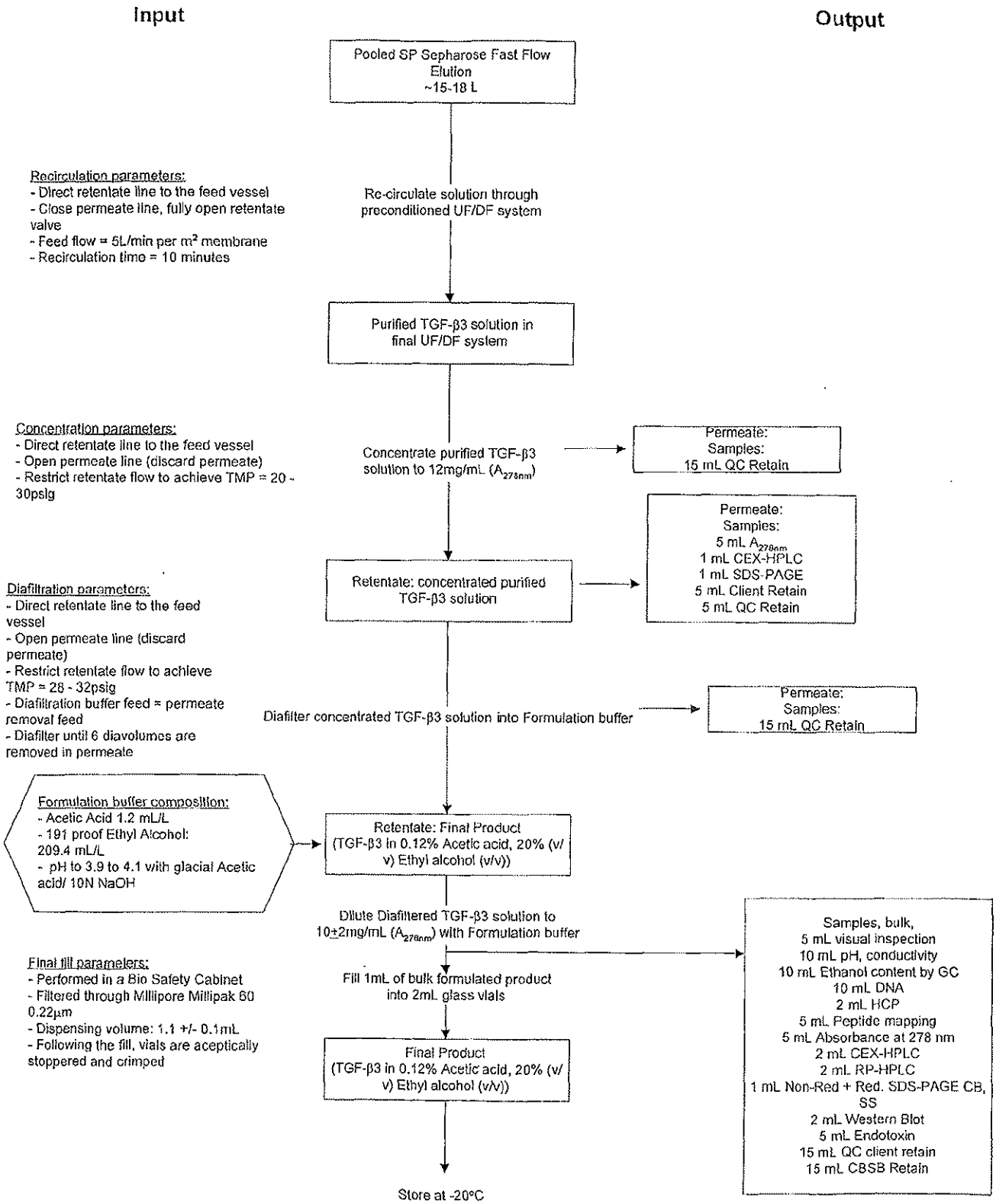

Pooled SP Sepharose Fast Flow Elution
Re-circulate solution through preconditioned UF/DF system
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Feed flow = 5L/min per m² membrane
- Recirculation time = 10 minutes
Purified TGF-β3 solution in final UF/DF system
Concentrate purified TGF-β3 solution to 10mg/mL (A_{278nm})
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Restrict retentate flow to achieve TMP = 20 - 30psig
Retentate: concentrated purified TGF-β3 solution
Diafilter concentrated TGF-β3 solution into Formulation buffer
Diafiltration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Restrict retentate flow to achieve TMP = 28 - 32psig
- Diafiltration buffer feed = permeate removal feed
- Diafilter until 6 diavolumes are removed in permeate
Formulation buffer composition:
- Acetic Acid 1.2 mL/L (0.12% (v/v))
- Ethyl Alcohol: 200 mL/L (20%(v/v))
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
Retentate: Final Product (TGF-β3 in 0.12% Acetic acid, 20% (w/w) Ethyl alcohol (w/w))
Fill 1mL of bulk formulated product into 2mL glass vials
Final fill parameters:
- Performed in a Bio Safety Cabinet
- Filtered through Millipore Millipak 60 0.22µm
- Dispensing volume: 1.1 +/- 0.1mL
- Following the fill, vials are aceptically stoppered and crimped
Final Product (TGF-β3 in 0.12% Acetic acid, 20% (w/w) Ethyl alcohol (w/w))
Store at -20°C

FIG 29B


Input
Output
Pooled SP Sepharose Fast Flow Elution ~15-18 L
Recirculation parameters:
- Direct retentate line to the feed vessel
- Close permeate line, fully open retentate valve
- Feed flow = 5L/min per m² membrane
- Recirculation time = 10 minutes
Re-circulate solution through preconditioned UF/DF system
Purified TGF-β3 solution in final UF/DF system
Concentration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Restrict retentate flow to achieve TMP = 20 - 30psig
Concentrate purified TGF-β3 solution to 12mg/mL (A_{278nm})
Permeate: Samples: 15 mL QC Retain
Retentate: concentrated purified TGF-β3 solution
Permeate: Samples: 5 mL A_{278nm} 1 mL CEX-HPLC 1 mL SDS-PAGE 5 mL Client Retain 5 mL QC Retain
Diafiltration parameters:
- Direct retentate line to the feed vessel
- Open permeate line (discard permeate)
- Restrict retentate flow to achieve TMP = 28 - 32psig
- Diafiltration buffer feed = permeate removal feed
- Diafilter until 6 diavolumes are removed in permeate
Diafilter concentrated TGF-β3 solution into Formulation buffer
Permeate: Samples: 15 mL QC Retain
Formulation buffer composition:
- Acetic Acid 1.2 mL/L
- 191 proof Ethyl Alcohol: 209.4 mL/L
- pH to 3.9 to 4.1 with glacial Acetic acid/ 10N NaOH
Retentate: Final Product (TGF-β3 in 0.12% Acetic acid, 20% (v/v) Ethyl alcohol (v/v))
Dilute Diafiltered TGF-β3 solution to 10±2mg/mL (A_{278nm}) with Formulation buffer
Samples, bulk, 5 mL visual inspection 10 mL pH, conductivity 10 mL Ethanol content by GC 10 mL DNA 2 mL HCP 5 mL Peptide mapping 5 mL Absorbance at 278 nm 2 mL CEX-HPLC 2 mL RP-HPLC 1 mL Non-Red + Red. SDS-PAGE CB, SS 2 mL Western Blot 5 mL Endotoxin 15 mL QC client retain 15 mL CBSB Retain
Final fill parameters:
- Performed in a Bio Safety Cabinet
- Filtered through Millipore Millipak 60 0.22μm
- Dispensing volume: 1.1 +/- 0.1mL
- Following the fill, vials are aceptically stoppered and crimped
Fill 1mL of bulk formulated product into 2mL glass vials
Final Product (TGF-β3 in 0.12% Acetic acid, 20% (v/v) Ethyl alcohol (v/v))
Store at -20°C