

ZHT-4-008 U.F.

31-1-86

13

1. SM, ZHT-4-008 post 0.45 μ m filter and -40°C freezing.
2. Starting volume, post thawing, 410 ml.
65 mM NaCl added to ZHT and pH $\rightarrow$ 7.0 with 0.25 M HCl.
ZHT then filtered to 0.2 μ m. Gave a vol of 370 ml for U.F.
3. Preflush buffer: 100 mM NaCl
20 mM TSC
2.5 mM CaCl_2
10 mM Tris
pH $\rightarrow$ 7.0.
4. Concentration by $\sim 10\times$ followed by 3 vols. diafiltration with: 20 mM lysine
100 mM NaCl
20 mM TSC
2.5 mM CaCl_2
10 mM Tris
pH $\rightarrow$ 7.0.
5. Inl samples taken.
6. No-Air start and finish.

Details.

Time	Vol. (concentration)	Inlet pressure (psi)	Pump no.	Filtrate Rate ml/min
11.28 am		5 1/2	6 1/2 + Back pressure	3.5 ml
11.56	100 ml	"	"	
12.25	200 ml	"	"	
12.54	300 ml	"	"	
1.07	335 ml change to smaller cylinder for reservoir, momentary stop. on Restarting	7 1/2	6 + back pressure.	2.4
1.13	350	"	"	
	Diafiltration.	10d taken as 40 ml		
1.21	1/2 vol (20 ml)	7 1/2	6 + B.P.	
1.30	1	"	"	2.6
1.37	1 1/2	8	"	
1.46	2	7 1/2	"	
1.53	2 1/2	7 +	"	2.6
2.02	3	7		

Starting vol = 370 ml.
Final vol = 42 ml.

SNBTS DOCUMENT REQUEST.No:

2010/00045

FACTOR VII ASSAY REQUEST FORM (Q32)

DATE 6-2-86

T_E , expt of 5.2.86

[illegible]

* TE₁ appeared clotted - Supernatant assayed

DATE: 6.2.86

SIGNATURE: Elspeth Thomson

ZHT 4-008 x 5 concentration.

12.2.86.

1. SM, frozen ZHT 4-008. 65 mM NaCl added to ~300 ml then hand-filtered to 0.45 μ m. giving a starting vol of 287 ml (post SM samples) for UF.
2. Preflush : 100 mM NaCl
and 20 mM Tris
No AIR Start, 2.5 mM CaCl_2
20 mM Ergone
pH 7.0
3. Minitan was "decaked", after N44218 run. Small amounts of 'gel' were found on membranes.

UF details.

Time	vol.	Pressure	Pump.
1.35 pm	—	5	5 ³ / ₄ + Back pressure
1.55	50 ml	"	"
2.29	150 ml	5 ¹ / ₂	"
2.46	200 ml	6"	"
2.59	235 ml	7	"

Final vol, post No AIR (using preflush) finish = 49.5 ml.

2 ml samples taken throughout and sent to coag and QC for TP, Na^+ , Cl^- and osmolality.

5 ml fills (9) and 1 x 2.5 ml fill into 30 ml vials and dried on SMJR.

R+D.

230

FACTOR VIII ASSAY REQUEST FORM (Q32)DATE 18/2/86

2 stage Z.

SAMPLE	Q.C. NUMBER	ESTIMATE (U/ML)	RESULT (U/ML)
3HT 4008 X5000 SM 12.286		2	3.78
post 50 ml		3	4.12
post 150 ml		5	5.68
post 200 ml		8	9.59
post 235 ml		10	14.17
3HT 4008 X5000 2% Sucrose 12.286	Small clots	15 15	8.23
25 mM lysine	"	"	7.72
20% Sucrose + 25 mM NaCl	"	"	7.75
25 mM lysine + 25 mM NaCl	"	"	8.16

ml
used

NOTES:

Ron, Please put this in the 'VH' box file when you receive it.

NYU 27: Appears to be a gradual increase of fibrous material
part reacting from 50 to 80°C - see vials by Spiral mixer.

DATE: 18/2/86SIGNATURE: pp A. Ketchen

TE₁ preparation and ultrafiltration.

24-25.2.86.

TE₁ processed as far as step 6 on printed sheet as shown overleaf. No initial sample taken of TE₁.

Post step 6 : (a) 80 mM NaCl added and pH adjusted to 7.2 using 100 mM Tris base. Sample then taken (1st for COAG).

(b) Filtered to 1.2 μ m, no prefilter used.
Sample taken. SM then frozen overnight in -70°C.

25.2.86 (c) Sample taken post thawing
SM filtered to 0.45 μ m - no problems. (No prefilter used).
Starting vol = 500 ml.

Preflush for Minitan : 20 mM Tris
115 mM NaCl
20 mM TSC
5.5 mM CaCl₂
pH 7.2.

4. Diafiltration Buffer : 5 vol: 20 mM Tris
115 mM NaCl
20 mM TSC
2.5 mM CaCl₂
pH 7.2

Ultrafiltration.

Time	Vol.	Pressure psi	Pump.	Sample size
10.09	No AIR start (+20ml to vol = 520ml)	6	5 1/2 (No Back P).	2 1/2 ml
10.13	Tubing burst.			
10.20, 10.22	Refilled with preflush. No air restart (vol now $\approx$ 540 ml).			
11.02	120 ml concn.	6	5 1/2	2 1/2 ml.
11.49	240 ml	-	"	"
12.26	340 ml	"	"	"
1.07	446 ml	7	"	2 ml
1.27	Slight froth on reservoir	Ultra diafiltration step.		
1.49	1 vol cloudy.	7	5 1/2	2 1/2 ml
2.00	Froth seems to have cleared away. Still cloudy reservoir			
2.27	2 vol	7	5 1/2	2 1/2 ml
3.03	3 vol	8	-	"
3.40	4 vol	8	-	"
3.52	Fibrous 'bits' on side of cylinder.			
4.16	5 vol	8	5 1/2	2 1/2.

No AIR finish.

Starting vol = 500 ml
Final vol = 102 ml.

24-25-2866

6. Final retentate was filtered to 0.45 μ m through Sweeney filters. No prefilter used and some difficulty encountered as 3-4 filters were blocked and the final filled volume was 77 ml, starting from ~100 ml.
7. 5 ml fills and 1 x 2 ml into 30 ml vials and run on SATJR Auto tape no 5.

U.F.

230

5

FACITOR VIII ASSAY REQUEST FORM (Q32)DATE 25.2.86

TE, 24/25-2-86.

SAMPLE	Q.C. NUMBER	ESTIMATE (U/ML)	RESULT (U/ML)
post 80 ml NaCl addition 24.2.86	①	8	3.79
post 1.2 µm 24.2.86	②	6	3.29
post 1.2 µm 25.2.86	③	"	3.68
post 0.4 µm	④	"	3.61
UF concn post 120 ml	⑤	8-10.	6.88
concn 240 ml	⑥		5.20
concn 340 ml	⑦		7.85
Post 446 ml. DF S.M.	⑧	5x concn	13.07
UF 1 vol	⑨	"	13.29
2	⑩		13.27
3	⑪		11.31
4	⑫	FROZEN OVERNIGHT	10.99
5 NO AIR	⑬		11.57
Final fill post 0.45 µm	⑭		11.16

assayed
10/250assayed
10/50

NOTES:

DATE: 26.2.86SIGNATURE: Elsbeth Thomson

Ninitan characterization.

26-2-86.

Recirculating fluid was new recipe AIV buffer.

. Amicon double pump, tubing in front pump.

3. Had to swap gaskets from 1st to 2nd pkt of membranes to stop leaking.

4. "Second pack" ie that used in expts of post 6.3.85 up to 24.2.86 gave results in table 1.

5. "First pack" ie membranes used in expts 14.6 to 6.3.85 gave results in table 2.

6. On putting these two packs of membranes together to give Ninitan x2 the flows of filtrate etc are given in table 3.

Results.

TABLE 1.

Pressure psi.	Pump no. (BP = Back pressure).	Filtrate Rate ml/min	Retentate Rate. ml/min
$\frac{2}{E}$	4	1.5	733 - really too fast to measure.
$\frac{2}{E}$	$4\frac{1}{2}$	1.8	
2-3 ie $\frac{1}{ZERO}$	5	2.3	
4	$5+$	2.4	
5	$5\frac{1}{2}$	2.6	
6	6	3.0	
7	6+BP	3.4	
8	6+BP+	4.2	
9	6+BP++	4.5	
10	6+BP+++	4.6	

TABLE 2.

$\frac{2}{E}$	4	4.4	735 too fast
$\frac{2}{E}$	$4\frac{1}{2}$	5.8	
2-3	5	6.6	
4	$5\frac{1}{2}$	6.8	
5	6	7.6	
6	6+BP	10.0	
7	6+BP+	12.0	
8	6+BP++	13.2	
9	6+BP+++	15.0	
10	6+BP++++	16.2.	

Table 3. Minitem X2 with A IV

Pressure psi	Pump no.	Filtrate Rate ml/min
$\frac{2}{1/2}$	3 ⁺	6.2
$\frac{2}{1/2}$	3 $\frac{1}{2}$	10.4
2-3	3 $\frac{3}{4}$	13.2
4	4 ⁻	15.4
5	4	17.2
6	4 ⁺	20.0
7	4 $\frac{1}{2}$	23.2
8	4 $\frac{3}{4}$	28.0
9	5 ⁻	33.6
10	5 ⁺	42.0

From 27-2.86. Minitem X2 with JHT 4-008.

Pressure psi	Pump no.	Filtrate Rate ml/min
2-3	3 $\frac{1}{2}$	4.5
4	3 $\frac{3}{4}$	5
5 ⁺	4 ⁻	6.5
6-7	4	7.5
8-9	4 ⁺	6.5 - 7.0
10-10 ⁺	4 $\frac{1}{2}$	9.0

Have found some slight inconsistencies

Pressure/psi	Pump no.	ml/min
8	4 ⁺	7.0
8-9	4 ⁺	6.5
11	4 ⁺	8.5
10	4	6.5
10	4	5.7
12	4	11

ZHT 4-008

8 cassette Minitan trial.

27-2-86.

1. Minitan system containing 8 cassette (10,000) ie 2 pks. Dead volume measured using preflush to be 25-30ml (27ml).

2. S.M. ZHT-4008 with 20mM TSC and 2.5mM CaCl_2 , pH 7.4 as of 25-2-86. On thawing, ZHT appeared to require ~~clearly~~ clearing $\therefore$ centrifuged for 15 mins @ 3000 rpm and then filtered to 0.45 μm . Filtering without prefilter was difficult (15-20ml / filter) but with a prefilter was even worse (10ml / filter) Hence no prefilter used.

3. Preflush: 20 mM Tris, 115 mM NaCl, 20 mM TSC, 5.5 mM CaCl_2 , pH 7.0.

5. Diafiltration buffer: New recipe AIV. 5 vol.

Starting vol = finishing vol = 102 ml.

wet start with preflush

wet finish with AIV.

Details and characterization of Minitan x2 with ZHT.

Time	vol	Pump.	Inlet Pressure (psi)	Filtrate Rate ml/min
12.02	-	3 1/2	2-3	4.5
		3 3/4	4	5
		4	5	6.5
		4	6.7	7.5
		4+	8	7.0
12.21	1 vol	4+	8-9	6.5
		4 1/2	10-10+	9.0
12.35	2 vol	4	11	8.5
12.37	pump lowered to	4	to get pressure down to 10 psi	
12.43	3 1/2	4	10	5.7
1.26	4 vol + 70 ml	4	12	11
1.29	5 vol	-	-	-