

APPENDIX II

PREPARATION OF IMPROVED INTERMEDIATE PURITY FVIII (Z8)

BATCH NO: Z8-6-002

DATE: 28/7/86

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8. SAMPLING REGIME
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APPENDIX II

PREPARATION OF IMPROVED INTERMEDIATE PURITY FILLI (50)

BATCH NO: 28-0-002

DATE: 28/1/88

CONTENTS

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8.	SAMPLING REGIME
9.	OPERATING THE US SYSTEM

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1. ZINC PRECIPITATION/A10H3 ADSORPTION

- ** 1.1 Tris extract (pH 7.0) _____ l
- 1.2 Titrate slowly from pH _____ to 6.7
with 0.05N HCl
- Use vibro mixer
- Volume used _____ mls
- Total volume _____ l (V_1)
- 1.3 Slowly add 333.3 mls of 4mM Zn Ac solution/l (V_1)
_____ mls
- Use vibro mixer
- 1.4 Equilibrate 5 mins at R.T. with gentle mixing.
- 1.5 Slowly add 12.5ml A10H3 suspension/l (V_1)
_____ mls
- 1.6 Equilibrate 5 mins at R.T. with gentle mixing.
- 1.7 Centrifuge 10 mins R.T. 3000 rpm 6 L.
- 1.8 Wash out the pots with 500mls 1mM ZnAc solution.
- 1.9 Clarify the pooled Zn/A10H₃ supernatant through a U220 filter.
- 1.10 Wash out the U220 filter with the 500mls pot wash.
- * 1.11 Zn/A10H3 supernatant final volume _____ l (V_2)

2. FORMULATION

- 2.1 Use vibro mixing throughout.
- Add a mixture of citrate and calcium chloride to 30mM and 4mM respectively.
- 62.6mls/l (V_2) 0.5M Citrate _____ mls
- 8.1mls/l (V_2) 0.5M Calcium Chloride _____ mls

* Sample
** Urgent Sample

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SING PRECIPITATION/AI0H3 ADSORPTION

- 1.1. Titrate extract (pH 7.0) _____ ml
- 1.2. Titrate slowly from pH _____ to pH _____ with 0.05M HCl
- Use vibro mixer
- Volume used _____ ml
- Total volume _____ ml (V₁)
- 1.3. Slowly add 333.3 ml of 4M Zn Ac solution (V₂) _____ ml
- Use vibro mixer
- 1.4. Equilibrate 5 mins at R.T. with gentle mixing.
- 1.5. Slowly add 15.2ml AI0H3 suspension (V₃) _____ ml
- 1.6. Equilibrate 5 mins at R.T. with gentle mixing.
- 1.7. Centrifuge 10 mins R.T. 3000 rpm 8 L.
- 1.8. Wash out the pots with 500ml 1M ZnAc solution.
- 1.9. Clarify the pooled Zn\AI0H₃ supernatant through a US20 filter.
- 1.10. Wash out the US20 filter with the 500ml pot wash.
- 1.11. Zn\AI0H₃ supernatant final volume _____ ml (V₅)

FORMULATION

- 2.1. Use vibro mixing throughout.
- Add a mixture of citrate and calcium chloride to 30ml and 4ml respectively.
- 82.6ml (V₅) 0.5M Citrate _____ ml
- 8.1ml (V₅) 0.5M Calcium Chloride _____ ml

* Sample
** Diluent Sample

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- 2.2 Add sucrose to 3 l
30g sucrose/l (V_2) _____g
- 2.3 Add 130mM NaCl
7.6g NaCl/l (V_2) _____g
- 2.4 Adjust to pH 6.9 using 0.05 HCl
 Volume used _____mls

- ** 2.5 Clarify the formulated Zn/A10H3 super through an NBP cartridge
 wash out the centrifuge with 100mls DF buffer _____l (V_3)

3. ULTRAFILTRATION

- 3.1 Concentrate the Zn/A10H3 supernatant to 25 iu/ml using the stainless steel Pelicon/LP20A pump system.

Total filtrate to be removed: _____l

N.B. UF Dead Volume

- ** 3.2 Final post conc retentate volume _____l (V_4)

- 3.3 Measure out 3 volumes ($3 \times V_4$) of diafiltration buffer.

- 3.4 Diafilter the concentrate against the measured volume of diafiltration buffer at R.T.

Final post DF volume _____l (Sample)

- 3.5 Adjust final volume in the UF apparatus to give 25iu/ml according to last urgent sample.

4. STERILE FILLING AND DISPENSING

- 4.1 Clarify the post DF material through an NBP cartridge.

- * 4.2 Sterile filter the clarified material through an NRP cartridge and wash out the cartridge with 100 ml DF buffer.

* Sample

** Urgent Sample

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3.1. Add sucrose to 3.1
 100 sucrose (V₂)
 Add 130mm NaCl
 3.2. 1.60 NaCl (V₂)
 Adjust to pH 6.0 using 0.07 N HCl
 Volume used _____ ml
 3.3. Clarify the formulated ZnVA10H3 super through an NRP cartridge
 wash out the cartridge with 100ml of buffer (V₃)

ULTRAFILTRATION

3.4. Concentrate the ZnVA10H3 supernatant to 25 ml/ml using the stainless steel Pelicon/P20A pump system.
 Total filtrate to be removed: _____ l
 H.B. UF Dead Volume
 Final post conc retentate volume _____ l (V₄)
 3.5. Measure out 3 volumes (3 x V₄) of dialysis buffer.
 3.6. Dialyze the concentrate against the measured volume of dialysis buffer at R.T.
 Final post UF volume _____ l (2 samples)
 3.7. Adjust final volume in the UF apparatus to give 250ml according to last urgent sample.

STERILE FILLING AND DISPENSING

4.1. Clarify the post UF material through an NRP cartridge.
 4.2. Sterile filter the clarified material through an NRP cartridge and wash out the cartridge with 100 ml of buffer.

1 Sample
 ** Urgent Sample

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- 4.3 Dispense 15ml aliquots into 65ml vials with bungs in freeze drying position.

Total number of vials _____

- 4.4 Store 20 vials in the -40 °C cold room and pass the remaining trays on for freeze drying.

5. FREEZE DRYING

- 5.1 Freeze the vials for at least 9 hours at -50 °C shelf temperature in the SM 200.

- 5.2 Freeze dry the vials controlling the following parameters.

Primary Drying

<u>Time</u>	<u>Shelf Temp.</u>	<u>Vacuum</u>
24 hrs	-30 °C	0.1m Barr
12 hrs	-20 °C	0.1m Barr

Secondary Drying

<u>Time</u>	<u>Shelf Temp.</u>	
24 hrs	+25 °C	0.10m Barr and then uncontrolled when product temperature reaches +10 °C

- 5.3 Stopper cap and seal the vials as normal.
- 5.4 Pass 10 vials to R & D and heat treat the remainder.

6. HEATING

- 6.1 Heat _____ vials in the Pickstone Oven at 80 ° for 72 hours

At 24 hours remove _____ vials

At 48 hours remove _____ vials

Remove the remainder at 72 hours

- 6.2 On removal from the oven treat the heated vials as a normal product batch.

APPENDIX II

- 4.3 Dispense 17ml aliquots into 67ml vials with buds in freeze drying position.
- Total number of vials _____
- 4.4 Store 20 vials in the -40°C cold room and pass the remaining trays on for freeze drying.

FREEZE DRYING

- 5.1 Freeze the vials for at least 8 hours at 20°C and freeze temperature in the 2M 200.
- 5.2 Freeze dry the vials controlling the following parameters.

Primary Drying

Time	Shelf Temp.	Vacuum
15 hrs	20°C	0.1m Bar
24 hrs	-30°C	0.1m Bar

Secondary Drying

Time	Shelf Temp.
24 hrs	+22°C

0.10m Bar and then uncontrolled when product temperature reaches +10°C

- 5.3 Stopper can and seal the vials as normal.
- 5.4 Pass 10 vials to R & D and heat treat the remainder.

HEATING

- 6.1 Heat _____ vials in the Brockstone Oven at 80° for 15 hours
- At 24 hours remove _____ vials
- At 48 hours remove _____ vials
- Remove the remainder at 75 hours
- 6.2 On removal from the oven treat the heated vials as a normal product batch.

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7. REAGENTS

- 7.1 4mM ZnAc 20mM Tris pH 6.7
- 7.2 1mM ZnAc 30mM Tris pH 6.7
- 7.3 0.05N HCl
- 7.4 0.5M Sodium Citrate
- 7.5 0.5M Calcium Chloride
- 7.6 Diafiltration (DF) buffer (20mM Tris; 130mM; 30mM TSC; 4mM CaCl₂; 3% Sucrose; pH 6.9)
- 7.7 0.1N NaOH
- 7.8 Saline
- 7.9 Hypochlorite

8. SAMPLING REGIME

At each point take :

3 x 2ml samples into polypropylene tubes and 1 x 10ml sample into a polypropylene centrifuge tube.

N.B. At sample points 2.5 and 3.2 take an additional 2ml sample for urgent assay by coagg.

9. OPERATION OF PELICON/LP20A UF SYSTEM

Date: 28/7/86 Cartridge Type: _____

No. of Cartridges: _____

- 9.1 Connect pump to Pelicon and run Pelicon outlet to waste.
- 9.2 Flush out the hypochlorite storage solution with 10 litres of RT PFW. Dead volume 700mls.
- 9.3 Using 3 litres of DF Buffer pass 1 litre through the system to waste and then recirculate with the remaining 2 litres.
- 9.4 Without draining the DF Buffer from the system connect the Retentate inlet line and Retentate outlet line to the Zn/A10H3 super.

- α.1 η εφευρετική της και βεβαιώνει αυτή η ημερομηνία της σύλληψης της με την ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
- α.2 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
- α.3 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
- α.4 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.

Ημερομηνία έκθεσης: _____

Όνομα: _____ Στοιχεία: _____

ΠΕΡΙΓΡΑΦΗ ΤΗΣ ΕΡΓΑΣΙΑΣ

Η εργασία αυτή αφορά στην:

1. ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.

2. η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.

3. η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.

ΣΥΜΠΕΡΙΛΗΨΗ

- 1.1 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
- 1.2 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
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- 1.5 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
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- 1.8 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.
- 1.9 η ημερομηνία της διενέργειας των εργασιών που αναφέρονται στην παρούσα έκθεση.

ΣΗΜΕΙΩΣΕΙΣ

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- 9.5 Begin concentrating the Zn/AlOH₃ super using 15 psi air supply to the pump and no back pressure. (Inlet pressure 10 psi).
- 9.6 Bring up the back pressure to obtain the required filtrate rate - do not allow the inlet pressure to exceed 20 psi.
- 9.7 Remove the required volumes of filtrate and diafiltrate.
- 9.8 On completion of diafiltration flush the system with 10 litres of saline using 20 psi air supply and no back pressure.
- 9.9 Using 2 litres of 0.1N NaOH flush 1 litre to waste and then stand the system in the remaining 1 litre of 30 mins.
- 9.10 Flush out the NaOH with RT PFW until pH <8.0.
- 9.11 Recirculation 4 litres of a 1000 ppm dilution of stock hypochlorite for 15 mins.
- 9.12 Connect Pelicon outlet and inlet lines to close the system and isolation LP20A pump.
- 9.13 Flush the pump with 5 litres of RT PFW; drain and store dry.

APPENDIX II

- 2.5 Begin concentrating the NaOH solution using 12 psi air supply to the pump and no back pressure. (Inlet pressure 10 psi).
- 2.6 Bring up the back pressure to obtain the required filtrate rate - do not allow the inlet pressure to exceed 20 psi.
- 2.7 Remove the required volume of filtrate and distillate.
- 2.8 On completion of distillation flush the system with 10 litres of saline using 20 psi air supply and no back pressure.
- 2.9 Using 5 litres of 0.1N NaOH flush 1 litre to waste and then stand the system in the remaining 1 litre of 20 mins.
- 2.10 Flush out the NaOH with RT FW until pH < 8.0.
- 2.11 Recirculation 4 litres of a 1000 ppm dilution of stock pyochlorite for 12 mins.
- 2.12 Connect isolation outlet and inlet lines to close the system and isolation P250A pump.
- 2.13 Flush the pump with 2 litres of RT FW; drain and store dry.