Manufacturing Batch Record **MBR[redacted]**

Title: - FORMULATION OF [redacted] CONJUGATE CONCENTRATE

Identifying Number: [redacted] **Batch Number:**

All operators involved in this formulation must add their name, signature and identifying initials to the Table below.

NAME	SIGNATURE	INITIALS

QC MBR REVIEW

NAME	SIGNATURE	DATE

Title: - FORMULATION OF [redacted] CONJUGATE CONCENTRATE

Identifying Number: [redacted] **Batch Number:**

1. RAW MATERIALS and CONSUMABLES

Description	Supplier	Cat. Code / Part No.	Lot No.	Expiry Date
0.01M Potassium Phosphate 0.09% Azide (≥20 Litres)	FBL	SOP032 Buffer 14		
0.5M Sodium Hydroxide solution (≥16 Litres)	FBL	SOP032 Buffer 13		
Dipotassium hydrogen orthophosphate anhydrous	VWR	29619		
Potassium dihydrogen orthophosphate	VWR	10203		
Sodium chloride	VWR	10241		
Biotin-XX-NHS	[redacted]	[redacted]		
Avidin	[redacted]	[redacted]		
4-Hydroxy-azobenzene-2'-carboxylic acid (HABA)	SAF	H5126		
0.2µ 2″ Sartobran filter	Sartorius	5231307H7		N/A
Sartopure PP2 filter	Sartorius	5591303P5		N/A
0.2µ Sartobran 300 filter	Sartorius	5231307H5		N/A
0.2µ Sartolab P filter	Sartorius	18052		N/A
0.45µ SuporCap – 50 filter	PALL	12993		N/A
Vials	[redacted]	[redacted]		N/A
Bungs	[redacted]	[redacted]		N/A
Caps	[redacted]	[redacted]		N/A

2. EQUIPMENT

Description	SOP	Serial No.
Biosafety Cabinet	SOP006	
Balances	SOP007	
Magnetic stirrer	SOP008	
Vortex mixer	SOP009	
Roller mixer	SOP010	
Overhead stirrer	SOP011	
Pipette (≤200µl)	SOP012	
Pipette (>200µl)	SOP012	
Fridge / freezer	SOP013	
Thermometer	SOP014	
UV Spectrophotometer	SOP016	
pH meter	SOP017	
Water purification system	SOP018	
Watson-Marlow dispenser	SOP019	
Filter integrity tester	SOP020	
Peristaltic pump	SOP021	
AKTA chromatography system	SOP022	
Cooled incubator	SOP025	

2.1 Equipment Calibration

	Signature
Section 2 Operator	

2.1.1 Balance calibration

	Record	Check	Date
Balance Serial No.		N/A	
Serial No. of calibrated weights		N/A	
Recorded mass of 100g weight		N/A	
Pass / Fail (99.5 – 100.5g)			
Recorded mass of 10g weight		N/A	
Pass / Fail (9.95 – 10.05g)			
Recorded mass of 1g weight		N/A	
Pass / Fail (0.995 – 1.005g)			
Recorded mass of 10mg weight		N/A	
Pass / Fail (9.95 - 10.05mg)			

3. BUFFER FORMULATION AND COLUMN EQUILIBRATION

3.1. Formulation of 0.1M Phosphate buffer pH 7.2, 0.15M NaCl (PBS)

	Signature
Section 3 Operator	

	Check	Date
Line Clearance		

3.1.1. Weigh the listed reagents into a clean vessel.

	Record	Requirement	Balance ID
Water from a validated source		40.00Kg	
Dipotassium hydrogen orthophosphate		476g	
Potassium dihydrogen orthophosphate		100g	
Sodium Chloride		352g	

3.1.2. Mix by overhead stirring for 25 - 35 minutes post-visual dissolution.

Visual inspection	Time of inspection	Time mixing stopped	Elapsed time (25 - 35 minutes)	Calculation Check
Clear				

3.1.3. Check the pH of the buffer. If the pH is outside of the range 7.1 – 7.3, discard the buffer.

Temperature (18 - 22°C)	pH (7.1 - 7.3)	Check

3.1.4. Filter the PBS through a water wetted 0.2µ 2″ Sartobran filter into a sanitised vessel.

Filter type	Water flush (≥2000ml)	Product flush (≥200ml)
0.2µ 2″ Sartobran filter		

3.1.5. Label the solution as follows: -

Label: -	Record
In Process PBS Buffer	
Prep. Date	
Expiry Date (3 days after Prep.)	
Store at Room Temperature	

3.2. Formulation of 0.1M Phosphate buffer pH 7.4

3.2.1. Weigh the listed reagents into a clean vessel.

	Record	Requirement	Balance ID
Water from a validated source		100g	
Dipotassium hydrogen orthophosphate		1.19g	
Potassium dihydrogen orthophosphate		0.250g	

3.2.2. Mix by magnetic stirring for 10 - 30 minutes post-visual dissolution.

Visual inspection	Time of inspection	Time mixing stopped	Elapsed time (10 - 30 minutes)	Calculation Check
Clear				

3.2.3. Check the pH of the buffer. If the pH is outside of the range 7.3 – 7.5, discard the buffer.

Temperature (18 - 22°C)	pH (7.3 - 7.5)	Check

3.2.4. Label the solution as follows: -

Label: -	Record
In Process 0.1M Phosphate Buffer pH 7.4	
Prep. Date	
Expiry Date (3 days after Prep.)	
Store at Room Temperature	

3.3. Formulation of 2.5mM Sodium Hydroxide

3.3.1. Weigh the listed reagents into a clean vessel.

	Record	Requirement	Balance ID
Water from a validated source		99.5g	
0.5M NaOH Solution		0.50g	

3.3.2. Mix by magnetic stirring for 10 - 30 minutes.

Time mixing started	Time mixing stopped	Elapsed time (10 - 30 minutes)	Calculation Check

3.3.3. Label the solution as follows: -

Label: -	Record
In Process 2.5mM NaOH Solution	
Prep. Date	
Expiry Date (3 days after Prep. Date)	
Store at Room Temperature	

3.4. Equilibration of Sephadex Column

3.4.1. Connect the Sephadex Index 100/60 column reserved for [redacted].

	Record	Requirement
Sephadex Index 100/60 column connected		✓

3.4.2. Connect the ports of the injection valve (Valve 1) as follows:

1 Out to column
2 In from loading pump (Pump B)
3 Out to conjugate reservoir
4 Waste
5 Waste
6 Waste
7 In from running pump (Pump A)

	Record	Requirement
Ports connected as above		✓

3.4.3. Program Method 1 listed and attach a print out below: -

METHOD NO. 1

0.0	VALVE.POS	1.3
0.0	WASH A.B.	1.1
0.0	CONC. %B	0.0
0.0	VALVE.POS	1.1
0.0	ML/MIN	32
450.0	CONC %B	0.0

If different valve numbers are used from those shown, record the number and state why.

Valve Record / Reason (if applicable)

3.4.4. Add ≥ 18 litres of PBS to the reservoir for Pump A and Pump B and run Method 1 to equilibrate column.

	Record	Requirement
Pump A & B to ≥18 Litres of PBS		✓
Method 1 Run		✓

4. BIOTINYLATION

4.1. Equilibration of [redacted]

	Signature
Section 4 Operator	

	Check	Date
Line Clearance		

Remove the [redacted] from the fridge and store at room temperature for ≥15 hours.

Time [redacted] removed from Fridge	Time [redacted] used (Section 4.2.1)	Elapsed time (≥15 hours)	Calculation Check

4.2. Formulation of 50mg/ml [redacted] Solution

4.2.1. Measure the listed materials into a clean glass vessel.

	Record	Requirement	Balance ID
PBS		800g	
[redacted]		40.00g	

4.2.2. Mix by magnetic stirring in an incubator at 18 - 22°C until the [redacted] has dissolved (<2 hours). Continue stirring at 18 - 22°C until Section 4.3.3.

	Record	Requirement	Check
Time mixing started		N/A	N/A
Time mixing stopped		N/A	N/A
Elapsed time		<120 minutes	
Dissolution check		Visual solution	
Temperature		18 - 22°C	

4.3. Conjugation reaction

4.3.1. Remove the Biotin-XX-NHS from the fridge and store at room temperature for ≥30 minutes. Ensure that the batch purity of the Biotin-XX-NHS has been supplied by [redacted] and record below.

	Record	Requirement	Check
Biotin-XX-NHS purity	(**A**)	N/A	
Time Biotin-XX-NHS removed from fridge		N/A	N/A
Time Biotin-XX-NHS used		N/A	N/A
Elapsed time		≥30 minutes	

4.3.2. To three decimal places, determine the amount of Biotin-XX-NHS to add to the [redacted] Solution (Section 4.2.2) using the formulae below.

Weight Biotin-XX-NHS (**B**) g = [redacted] / (**A**)

(**B**) = [redacted]

(**B**) = _____g

4.3.3. Weigh (**B**) g of Biotin-XX-NHS into a clean glass vial and add to the stirred [redacted] solutions (Section 4.2.2). Record the time of addition. Continue to stir the solution at [redacted] °C for [redacted] minutes. At the end of the mixing time, the solution must be filtered immediately (Section 4.4.3).

	Record	Requirement	Balance ID
Biotin-XX-NHS		(**B**) g	
Time Biotin-XX-NHS added [redacted]		N/A	N/A
Temperature		[redacted] °C	N/A
Time conjugate mixture filtered (Section 4.4.3)		N/A	N/A
Elapsed time		[redacted] minutes	N/A

4.4. [redacted] Purification

NB. Method 2 must be started at least 5 minutes before filtration of the [redacted] solution.

4.4.1. Program Method 2 listed and attach a print out below: -

METHOD NO. 2

0.0	VALVE.POS	1.3
0.0	WASH A.B	1.1
0.0	VALVE.POS	1.1
0.0	CONC %B	0.0
0.0	ML/MIN	32
0.0	CM/MIN	0.2
0.0	ALARM	1.0
0.5	ALARM	0.0
1.0	HOLD	
1.0	CONC %B	0.0
1.0	CONC %B	100
1.0	ML/MIN	32
6.0	VALVE.POS	1.2
6.0	CLEAR DATA	
6.0	MONITOR	1
6.0	LEVEL %	5.0
6.0	MIN/MARK	2.0
6.0	INTEGRATE	1
30.0	CONC %B	100
30.0	CONC %B	0.0
30.0	VALVE.POS	1.1
156.0	INTEGRATE	0
156.0	PRT PK	1.99
156.0	CONC %B	0.0

If different valve numbers are used from those shown, record the number and state why.

Valve Record / Reason (if applicable)

4.4.2. Ensure Pump A reservoir contains ≥ 6 litres of PBS and Pump B inlet is in air (to empty pump B prior to loading). Set the 280 nm absorbance monitor to 2 absorbance scale. Adjust the chart recorder pen to 5% base line. Run Method 2. After 1 minute the program will HOLD. The sample can be injected at any stage after this.

	Record	Requirement
Pump A inlet to ≥6 Litres of PBS		✓
Pump B inlet to air		✓
280nm set to 2 absorbance scale		✓
Time Method 2 Commenced		N/A

4.4.3. Pre-flush a Sartopure PP2 filter with ≥1.5L of water, followed by ≥500ml of PBS. Filter the solution from Section 4.3.3 into a clean and sanitised glass container.

Filter type	Water flush (≥1500ml)	PBS flush (≥500ml)
Sartopure PP2 filter		

4.4.4. Add the to Pump B reservoir.
[N.B. if <800ml, make up to volume with extra PBS).
Direct Valve 1, port 3 back into the conjugate reservoir, in order to flush all sample on to the column.
Press "CONT" to inject the sample onto the column and start purification.

	Record	Requirement
added to Pump B reservoir		✓
Time program continued		N/A

4.4.5. Manually collect the first major peak as it elutes (corresponding to) into a sanitised 2L vessel.

	Record	Requirement	Check
collected		✓	

4.4.6. Attach the labelled, signed and dated AKTA trace and integration printout to the following page.

AKTA Trace

4.5. Column Sanitisation

4.5.1. Connect the ports of the injection valve (Valve 1) as follows:

1 Out to column
2 Waste
3 Waste
4 Waste
5 Waste
6 Waste
7 In from Pump A/B

	Record	Requirement
Ports connected as above		✓

4.5.2. Program Method 3 listed and attach a print out below: -

METHOD NO. 3

0.0	VALVE.POS	1.3
0.0	WASH A.B.	1.1
0.0	CONC %B	0.0
0.0	VALVE.POS	1.1
0.0	ML/MIN	39.2
240.0	ML/MIN	0.0
300.0	CONC %B	0.0
300.0	CONC %B	100
300.0	ML/MIN	39.2
660.0	CONC %B	100

If different valve numbers are used from those shown, record the number and state why.

Valve Record / Reason (if applicable)

4.5.3. Add ≥ 12 litres of 0.5 M NaOH to Pump A reservoir, ≥ 18 litres of 0.1 % azide to Pump B reservoir and run Method 3 to sanitise the column.

	Record	Requirement
Pump A to ≥12 Litres of 0.5 M NaOH		✓
Pump B to ≥18 Litres of buffered azide		✓
Method 3 Run		✓

5. PROCESSING OF [redacted]

	Signature
Section 5 Operator	

	Check	Date
Line Clearance		

5.1. Terminal filtration and dilution to [redacted]

5.1.1. Pre-flush a 0.2µ Sartobran 300 filter with ≥900ml of water, followed by ≥300ml of PBS. Filter the [redacted] from Section 4.4.5 into a clean and sanitised pre-weighed 10L vessel.

Filter type	Water flush (≥900ml)	PBS flush (≥300ml)
0.2µ Sartobran 300 filter		

Weight of 10L vessel	Weight of vessel plus [redacted]	Weight of [redacted]	Calculation Check	Balance ID
		(**C**)		

5.1.2. Flush the filter with water and integrity test (diffusion and bubble point test). Attach the integrity test printout to the MBR.

	Record	Requirement	Check
Water Flush Volume		500 – 1500ml	N/A
Diffusion test		<1.5 ml/min	
Bubble Point		≥3.2 Bar	
PASS / FAIL		PASS	

5.2. Concentration Determination

5.2.1. In triplicate, add 150 µL of the [redacted] to 2.85 mL PBS and vortex mix for ≥20 seconds.

	Record			Requirement
	Sol^{n} 1	Sol^{n} 2	Sol^{n} 3	
[redacted]				150µL
PBS				2.85ml
Vortex mixed ≥20 seconds				✓

5.2.2. Determine the absorbance of the diluted conjugates at 280nm by using 10mm path length silica cells and PBS as the blank. Calculate the mean Abs280nm of the three diluted conjugate solutions.

	Record	Requirement	Check
Abs 280nm Sol^{n} 1		N/A	
Abs 280nm Sol^{n} 2		N/A	
Abs 280nm Sol^{n} 3		N/A	
Mean Abs 280nm	(**D**)	N/A	

5.2.3. Determine the concentration using the formulae below: -

	Record mg/ml	Requirement	Check
	(**E**)	N/A	

5.2.4. Determine the yield of the formula below: -

	Record	Requirement	Check
Yield = (**E**) x (**C**)	(**F**)	N/A	
% Yield = (**F**)/400		N/A	

5.3. Dilution to and Addition

5.3.1. Determine the total weight of the solution using the formulae below: -

	Record g	Requirement	Check
Weight of mg/ml Sol^{n} = (**F**)/	(**G**)	N/A	

5.3.2. Determine the weight of PBS required to dilute the (Section 5.1.1) to mg/ml using the formulae below: -

	Record G	Requirement	Check
Weight PBS required = (**G**) - (**C**)	(**H**)	N/A	

5.3.3. Determine the weight of required to prepare a % solution using the formulae below: -

	Record g	Requirement	Check
Weight required = (**G**) x	(**I**)	N/A	

5.3.4. Filter ≥6L of PBS through a water wetted 0.2µ Sartolab P filter into a sanitised vessel.

Filter type	Water flush (≥200ml)	PBS flush (≥10ml)
0.2µ Sartolab P filter		

5.3.5. Flush the filter with water and integrity test. Attach the integrity test printout to the MBR.

	Record	Requirement	Check
Water Flush Volume		400 - 600ml	N/A
Bubble Point		≥3.2 Bar	
PASS / FAIL		PASS	

5.3.6. Measure (**H**) g of the filtered PBS buffer and (**I**) g of [redacted] and add to the [redacted] from Section 5.1.1.

	Record	Requirement	Balance ID
Filtered PBS Buffer		(**H**) g	
[redacted]		(**I**) g	
Added to [redacted] from Section 5.1.1		✓	N/A

5.3.7. Mix by magnetic stirring for ≥15 minutes.

Time mixing started	Time mixing stopped	Elapsed time (≥15minutes)	Calculation Check

5.3.8. Label the solution as follows: -

Label: -	Record
[redacted]	
[redacted]	
Lot No.	
[redacted]	
Prep. Date	
Expiry Date [redacted]	
Store at 2 - 8°C	

5.4. Process Control: - Biotin Determination

5.4.1. Weigh 5 - 7 mg of HABA into a glass vial of at least 15ml capacity.

	Record mg	Requirement	Balance ID
HABA	(**J**)	5 – 7mg	

5.4.2. Using the formulae below, determine the volume of 2.5mM NaOH solution required and added to the HABA.

	Record	Requirement	Calculation Check
Volume of 2.5mM NaOH = (**J**)/0.6 ml		N/A	
2.5mM NaOH added to HABA		✓	N/A

5.4.3. Mix by vortexing for ≥20 seconds, followed by roller mixing for ≥30 minutes. Filter the solution through a 0.45µ acrodisc into a glass vial.

	Record	Requirement
Vortex mixed		≥20 seconds
Time roller mixing started		N/A
Time roller mixing stopped		N/A
Elapsed time		≥30 minutes
Solution filtered through Acrodisc		✓

5.4.4. Label the solution as follows: -

Label: -	Record
In Process 2.5mM HABA Solution	
Prep. Date / Time	
Expiry Date / Time (8 hours after Prep.)	
Store at Room Temperature	

5.4.5. Weigh ≥5mg of Avidin into a glass vial of at least 15ml capacity.

	Record mg	Requirement	Balance ID
Avidin	(**K**)	≥5mg	

5.4.6. Using the formula below, determine the volume of 0.1M Phosphate Buffer pH 7.4 and 2.5mM HABA solution required and added to the Avidin.

	Record	Requirement	Check
Volume of 0.1M Phosphate Buffer pH 7.4 = (**K**) x 1.8 ml		N/A	
Volume of 2.5mM HABA solution = (**K**) x 0.2 ml		N/A	
Materials added to Avidin		✓	N/A

5.4.7. Roller mix for ≥15 minutes.

Time mixing started	Time mixing stopped	Elapsed time (≥15minutes)	Calculation Check

5.4.8. Label the solution as follows: -

Label: -	Record
In Process HABA/Avidin Solution	
Prep. Date / Time	
Expiry Date / Time (4 hours after Prep.)	
Store at Room Temperature	

5.4.9. Measure the listed reagents into suitable containers and vortex mix for ≥20 seconds

Label: - **Test** [redacted]	Record	Requirement
Formulated [redacted] (Section 5.3.8)		[redacted]
0.1M Phosphate Buffer pH 7.4		[redacted]
Vortex mixed		≥20 seconds

Label: - **Reference** [redacted]	Record	Requirement
Reference [redacted]		[redacted]
0.1M Phosphate Buffer pH 7.4		[redacted]
Vortex mixed		≥20 seconds

5.4.10. Measure the Abs of the HABA/Avidin solution at 500 nm, using 10 mm path length silica cells and water as the blank.

	Record	Check
Abs. HABA/Avidin solution @ 500nm	(**L**)	

5.4.11. Pipette 4 x 1.0 ml samples of the HABA/Avidin solution into suitable test tubes. Add 50µl of the Test too two of the HABA/Avidin solutions and 50µl of the Reference too the remaining two HABA/Avidin solutions. Vortex each sample for 20 - 30 seconds. Measure the Abs_{500} & Abs_{600}, 10 (± 0.5) minutes after addition.

Test	Record	Check
Solution 1. Abs_{500} - Abs_{600}		
Solution 2. Abs_{500} - Abs_{600}		
Mean Abs_{500} - Abs_{600}		

Reference	Record	Check
Solution 1. Abs_{500} - Abs_{600}		
Solution 2. Abs_{500} - Abs_{600}		
Mean Abs_{500} - Abs_{600}		

5.4.12. Using the Formulae below, determine the for the Test and Reference solutions.

ratio = $[(\mathbf{L} \div 1.05) - (\text{Mean } Abs_{500} - Abs_{600})] \times$

	Record	Specification	Check
ratio for Test solution			
ratio for Reference			

5.5. Dispensing and Labelling

NB. Dispensing must be performed in the laminar flow cabinet.

5.5.1. Program the Watson-Marlow dispenser with the information listed below and calibrate the dispensing line with the [redacted] solution. Dispense the [redacted] solution, recording the weights of the first three and last three aliquots.

	Record	Requirement
Aliquot Dose Size		[redacted]
Specific Gravity		1.0
Dispense Interval (Sets dispenser to manual control)		0.0 seconds
Speed Setting		200RPM

	Record	Requirement	Balance ID	Check
Dispensing line calibrated with [redacted]		✓	N/A	N/A
Weight of First Aliquot		[redacted]		
Weight of Second Aliquot		[redacted]		
Weight of Third Aliquot		[redacted]		
Weight of Third Last Aliquot		[redacted]		
Weight of Second Last Aliquot		[redacted]		
Weight of Last Aliquot		[redacted]		
Mean Aliquot Size		[redacted]	N/A	
PASS / FAIL		PASS	N/A	

5.5.2. From the residual material in the dispensing line, dispense one ≥1ml sample into a sterile vial and send for bioburden analysis.

	Record	Requirement	Check
≥1ml sample dispensed to sterile vial		(✓)	N/A
Sample sent for bioburden analysis		(✓)	N/A
Bioburden Result		[redacted]	

5.5.3. Record the total number of aliquots dispensed: -

	Record	Requirement
No. of [redacted]		N/A

5.5.4. Label the vials as detailed below. Attach an example label to the Certificate of Analysis.

Label: -	Record
[redacted]	
Lot No.	
[redacted]	
Concentration = [redacted]	
Prep. Date	
Expiry Date [redacted]	
Aliquot Number (Bottle X of Y)	
Store at 2 - 8°C	

5.5.5. Store the vials at 2 - 8°C.

5.6. MBR REVIEW

MBR Complete (✓)	Operator	Date

Title: - FORMULATION OF [redacted] CONJUGATE CONCENTRATE

Identifying Number: [redacted] **Batch Number:**

Certification of Analysis

Component	[redacted]
Part Number	[redacted]
Batch Number	

	Specification	Result
Preparation Date	N/A	
Expiry Date	N/A	
Number of Aliquots	N/A	
Mean Aliquot Size	[redacted]	
Concentration	[redacted]	
[redacted] Ratio	[redacted]	
Bioburden	[redacted]	
Storage Temperature	2 - 8°C	

Example Label: -

Fleet Bioprocessing Operator Date

Signature: - _____ _____

QC Reviewer Date

Signature: - _____ _____