Manufacturing Batch Record **MBR####**

Title: FORMULATION OF AN ANTIBODY-BIOTIN CONJUGATE

Lot Number(s):

All unshaded boxes in this document should be completed.

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

NAME	SIGNATURE	INITIALS

INDEPENDENT MBR REVIEW

NAME	SIGNATURE	DATE

Title: FORMULATION OF AN ANTIBODY-BIOTIN CONJUGATE

Lot Number(s):

1. MATERIALS

Description	Supplier	Cat. Code / Part No.	Lot No.	Expiry Date
Antibody	######	######		
0.1M Potassium Phosphate pH 7.5 buffer	FBL	SOP032 Buffer 1		
0.1M Potassium Phosphate pH 7.4 buffer	FBL	SOP032 Buffer 4		
2.5mM Sodium Hydroxide solution	FBL	SOP032 Buffer 14		
0.5M Sodium Hydroxide solution	FBL	SOP032 Buffer 16		
0.01M Buffered Azide	FBL	SOP032 Buffer 19		
'Quench agent'	######	######		
'Biotin Reagent	######	######		
Anhydrous Dimethylformamide (DMF)	######	######		
2.6 x 20cm Sephadex G25M Column	######	######		
'Additive 1'	######	######		
'Additive 2'	######	######		
'Additive 3'	######	######		
0.2µ Minisart Filter	######	######		
0.2µ RC15 Minisart Filter	######	######		
0.45µ Minisart Filter	######	######		
0.45µ Acrodisc	######	######		N/A
Amber vials	######	######		N/A

2. EQUIPMENT

Description	SOP	Serial No.
Balances (≤100 grams)	007	
Balances (100 – 2000 grams)	007	
Balances (>2000grams)	007	
Magnetic stirrer	008	
Pipette (volumes ≤200µl)	012	
Pipette (volumes >200µl)	012	
Fridge-freezers	013	
Thermometer	014	
UV Spectrophotometer	016	
pH meter	017	
Water system	018	
Filter Integrity Tester	020	
FPLC system	022	
Cooled incubator	025	

3. SOLUTION FORMULATION

	Signature
Section 3 Operator	

	Check	Date
Line Clearance		

3.1 Formulation of Quench Solution

3.1.1 Weigh the listed reagents into a clean vessel.

	Requirement	Record	Balance ID	Check
'Quench agent'	10.0g			
0.1M Potassium Phosphate pH 7.5	100g			

3.1.2 Mix the solution with a magnetic stirrer for ≥5 minutes post visual dissolution.

Visual inspection	Time of inspection	Time mixing stopped	Elapsed time (≥5 minutes)
Clear			

3.1.3 Filter the solution into a sanitised vessel.

Filter type	Water flush (≥50ml)	Product flush (≥1ml)	Check
0.2µ Minisart			

3.1.4 Label the solution as follows: -

In Process Quench Solution	
Prep date	
Expiry date (3 days after prep date)	
Store at RT	

4. BIOTINYLATION

	Signature
Section 4 Operator	

	Check	Date
Line Clearance		

4.1 Desalt of Antibody

4.1.1 Thaw the antibody and mix by swirling.

Time thawing started	Time thawing stopped	Elapsed time	Hand Swirled (✓)

4.1.2 Weigh 25±0.5g of the antibody into a clean glass vial.

	Record	Requirement	Check
Weight of antibody		24.5 – 25.5g	

4.1.3 Connect the 2.6 x 20cm Sephadex G25M column to the FPLC unit.

	Record (✓)
2.6 x 20cm Sephadex G25M column connected	

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

1 Out to column
2 To Superloop
3 Injection port
4 Waste
5 Waste
6 To Superloop
7 In from Pump A/B mixer

	Record	Requirement	Check
Ports connected as above		✓	

4.1.5 Program Method 1 listed and attach a printout below: -

METHOD NO. 1

0.0	VALVE.POS	1.3
0.0	WASH A.B.	1.0
0.0	CONC. %B	0.0
0.0	VALVE.POS	1.1
0.0	ML/MIN	2.6
123.0	CONC %B	0.0

4.1.6 Add ≥1 litre of 0.1M phosphate pH 7.5 buffer to the reservoir for Pump A and run Method 1 to equilibrate column.

	Record (✓)
Pump A to ≥1 Litres of 0.1M phosphate pH 7.5 buffer	
Method 1 Run	

4.1.7 Set the FPLC unit as listed below: -

Superloop filled with 0.1M phosphate pH 7.5 buffer	
280 nm absorbance monitor set to 1 ABS	
Chart recorder pen adjusted to 5% base line	
Fraction collector set to collect 1 minute fractions	
Fraction collector filled with vials	

4.1.8 Program Method 2 listed and attach a printout below: -

METHOD NO. 2

0.0	VALVE.POS	1.2
0.0	CONC. %B	0.0
0.0	ML/MIN	2.6
0.0	CM/MIN	0.2
0.0	PORT.SET	6.1
40	PORT.SET	6.0
60	CONC. %B	0.0

4.1.9 Inject the antibody onto the Superloop and run Method 2.

	Record	Requirement
Antibody injected onto Superloop		(✓)
Method 2 Run		(✓)

4.1.10 Collect and pool all fractions corresponding to the first major peak. Mix by hand swirling and record the UV absorbance (280nm) of the solution. Determine the antibody concentration.

Fractions Selected	Fractions pooled (✓)	Hand Swirled (✓)	Abs_{280nm}	Concentration = (**A**) ÷ 1.4	Check
			(**A**)	mg/ml (**B**)	

4.1.11 Dispense a 10mg aliquot of the antibody.

	Record	Requirement	Calculation Check
Weight of solution required for 10mg aliquot /g	(**C**)	10 ÷ (**B**)	
Weight of aliquot		(**C**) g	N/A

4.2 Equilibrate the Biotin Reagent to 18 - 28°C.

Time on	Time off	Elapsed time (≥60 minutes)	Temperature

4.3 Formulate a 5.0mg/ml Biotin Reagent solution in anhydrous DMF.

Weight of Biotin Reagent (≥10mg)	Volume of DMF = (**D**) / 5 ml	Biotin Reagent dissolved (✓)	Balance ID	Check
(**D**)	ml			

4.4 Immediately add the required quantity of Biotin Reagent solution to the antibody aliquot. Incubate at 18 – 22°C for 110 – 130 minutes.

50 Equivalent of Biotin Reagent	Record	Requirement	Check
Vol. of Biotin Reagent solution added to antibody aliquot		360µl	

Time on	Time off	Elapsed time (110 - 130 mins)	Temperature (18 - 22°C)	Calculation Check

4.5 Quench the reaction at the end of the incubation time by adding the required quantity of quench solution and incubate.

Volume quench Sol^{n} added	Time on	Time off	Elapsed time (≥15 mins)	Temperature (18-22°C)	Calculation Check
147µl					

4.6 Conjugate Purification

4.6.1 Connect the 2.6 x 20cm Sephadex G25M column to the FPLC unit.

	Record (✓)
2.6 x 20cm Sephadex G25M column connected	

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

1 Out to column
2 To Superloop
3 Injection port
4 Waste
5 Waste
6 To Superloop
7 In from Pump A/B Mixer

	Record (✓)
Ports connected as above	

4.6.3 Set the FPLC unit as listed below: -

	Record (✓)
Pump A to ≥1 litre of 0.1M phosphate pH 7.5 buffer	
Superloop filled with 0.1M phosphate pH 7.5 buffer	
280 nm absorbance monitor set to 0.1 ABS	
Chart recorder pen adjusted to 5% base line	
Fraction collector set to collect 1 minute fractions	
Fraction collector filled with vials	

4.6.4 Program Method 3 listed and attach a printout below: -

METHOD NO. 3

0.0	VALVE.POS	1.2
0.0	CONC. %B	0.0
0.0	ML/MIN	2.6
0.0	CM/MIN	0.2
0.0	PORT.SET	6.1
40	PORT.SET	6.0
60	CONC. %B	0.0

4.6.5 Filter the conjugate solution through a 0.45µm Minisart filter. Collect the filtrate and inject onto the Superloop.

Filter type	0.1M Phosphate pH 7.5 buffer flush vol.	Check	Conjugate injected onto Superloop (✓)
0.45µ Minisart	≥5ml		

4.6.6 Run Method 3.

	Record (✓)	Check
Method 3 Run		

4.6.7 Pool vials corresponding to the first major peak into a clean pre-weighed vial.

	Record	Balance ID	Check
Fraction Nos. Selected		N/A	
Weight of empty vial	(**E**)		N/A
Weight of vial plus material	(**F**)		N/A
Weight of Antibody-biotin conjugate soln. = **F – E**	(**G**)	N/A	

4.6.8 Label the conjugate as detailed below.

In-Process Antibody-Biotin Conjugate	
Lot No	
Prep Date	
Store at 2 - 8°C	

5. PRODUCT MONITORING

	Signature
Section 5 Operator	

	Check	Date
Line Clearance		

5.1 Antibody Concentration

Record the UV absorbance (280nm) of the conjugate solution and determine the concentration.

Abs_{280nm}	Concentration = $(Abs_{280nm}) \div 1.4$	Yield = (**H** x **G**)	Calculation Check
	mg/ml (**H**)	mg	

5.2 Antibody : Biotin Incorporation Ratio

***** *Method removed from example MBR* ******

5.3 Addition of Additives

5.3.1 Re-weigh the vial containing the antibody-biotin conjugate.

	Record	Balance ID	Check
Weight of vial plus antibody-biotin conjugate	(**Q**)		N/A
Weight of antibody-biotin conjugate = **Q** – **E**	(**R**)	N/A	

5.3.2 Add the required quantities of additives to the conjugates.

	Record	Requirement	Balance ID
'Additive 1'		**R** x 10 (mg)	
'Additive 2'		**R** x 10 (µl)	N/A
'Additive 3'		**R** x 10 (µl)	N/A
Roller mix until visual dissolution		(✓)	N/A

5.4 Filter the conjugate.

Filter type	0.1M Phosphate pH 7.5 buffer flush vol.	Product flush vol.	Check
0.2µ Minisart RC15	≥5ml	2 Drops	

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

	Record	Requirement	Check
Water Flush Volume		≥10ml	N/A
Bubble Point		≥3.2 Bar	
PASS / FAIL		PASS	

5.6 Label the sample and attach an example label to the Certificate of Analysis.

Antibody-Biotin Conjugate	
Lot No	
Prep Date	
Concentration	
Biotin Incorporation Ratio	
Store at 2 - 8°C	

5.7 Column Sanitisation

5.7.1 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		✓

5.7.2 Program Method 4 listed and attach a printout below: -

METHOD NO. 4

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	2.6
82.0	ML/MIN	0.0
142.0	CONC %B	0.0
142.0	CONC %B	100
142.0	ML/MIN	2.6
265.0	CONC %B	100

5.7.3 Add ≥1 litre of 0.5 M NaOH to Pump A reservoir, ≥ 1 litre of buffered azide to Pump B reservoir and run Method 4 to sanitise the column.

	Record	Requirement
Pump A to ≥1 Litre of 0.5 M NaOH		✓
Pump B to ≥1 Litre of buffered azide		✓
Method 4 Run		✓

6. MBR REVIEW

MBR Complete (✓)	Operator	Date

Title: FORMULATION OF AN ANTIBODY-BIOTIN CONJUGATE

Lot Number(s):

Certification of Analysis

	Antibody-Biotin Conjugate
Batch Number	

Equivalents of Biotin Reagent used	Concentration (mg/ml)	Yield (mg)	Biotin : Antibody Incorporation Ratio
50			

	Record
Preparation Date	
Presentation Buffer: - 0.1M Potassium Phosphate pH 7.5 Buffer	
Additives Present: - 'Additive 1', 'Additive 2' and 'Additive 3'	
Filtered to 0.2µm	
Storage Temperature 2 - 8°C	

Example Label: -

Fleet Bioprocessing Operations Director Date

Signature: _____ _____

Fleet Bioprocessing Independent Reviewer Date

Signature: _____ _____