

Batch Record: GFP Production from *Escherichia coli* Upstream Process
GFP Lot Number _____

Record Keeping Standards:

For each step in the batch record: the operator of the task will enter their initials (each operator has their own unique set of initials) and the date in the appropriate section(s) of the batch record. Another operator must initial and date in the appropriate section of the batch record to verify that the task was completed per SOP. No operator will verify their own work at any point. "If you didn't document it, you didn't do it!"

Batch records will be completed in blue or black ball point pen ONLY, and must be legible.

Any errors on a batch record will be crossed out with a single line through the error with the initials of the operator and the date. Corrections will be written in next to the crossed out error.

Use the following format to record dates: DDMMYY. For July 10, 2006 use 10JUL06.

Use the 24 hour clock or "military time" to record time: 3:00pm would be written as 15:00.

Any and all deviations from a protocol or SOP, including abnormal results or retests performed, will be entered into the comments section at the end of each batch record. Be as detailed and specific as possible, include all steps taken before and/or after an abnormal reading, and provide an explanation for any deviations from a step.

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1. Prepare Media Broth Culture Broth: Luria-Bertani (LB) Broth, Ampicillin (0.09mg/mL), Arabinose (1.8mg/mL)		
Weigh out approximately 4g of LB Broth premix. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Weigh out approximately 0.36g of arabinose powder. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Add LB broth premix and arabinose to a clean 500mL shake flask. Flask I.D. number: _____	Operator/Date	Verifier/Date
Measure about 200mL of deionized water using a 250mL graduated cylinder and add it to the 500mL shake flask. Stir to dissolve. Volume of DI water measured: _____ mL	Operator/Date	Verifier/Date
Measure approximately 100mL of the LB/ARA broth using the 250mL graduated cylinder and transfer it to the 125mL glass bottle. Volume of LB Broth transferred from shake flask to bottle: _____ mL	Operator/Date	Verifier/Date
Autoclave per autoclave SOP for 20 minutes at 121°C. Autoclave I.D. #: _____ Time: _____ minutes Temperature: _____ °C Pressure: _____ psi	Operator/Date	Verifier/Date
Comments: 	Operator/Date	Verifier/Date

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2. Prepare Culture Agar Luria-Bertani (LB) Agar, Ampicillin (0.09mg/mL), Arabinose (1.8mg/mL)		
Weigh out approximately 35g of LB Agar (premix): Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Weigh out approximately 1.8g of arabinose powder: Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Measure approximately 1L of deionized water using a 1L graduated cylinder and add to the 2L Erlenmeyer flask. Stir to mix. Volume of DI water measured: _____ L	Operator/Date	Verifier/Date
Autoclave per autoclave SOP for 20 minutes at 121°C. Autoclave I.D. #: _____ Time: _____ minutes Temperature: _____ °C Pressure: _____ psi	Operator/Date	Verifier/Date
Remove from autoclave when pressure reaches <5psi and <80°C. Cool in a 55°C water bath.	Operator/Date	Verifier/Date
Comments: 	Operator/Date	Verifier/Date

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3. Prepare Ampicillin Solution and Add to Media Broth and Agar		
Ampicillin Stock solution: 6mg/mL		
Weigh out approximately 0.120g of ampicillin powder and place in small beaker. Manufacturer: _____ Catalog number: _____ Lot number: _____ Expiration date: _____ Amount weighed: _____ grams	Operator/Date	Verifier/Date
Measure approximately 20mL deionized water with a 25mL graduated cylinder and add to the beaker containing the ampicillin. Swirl to dissolve. Volume of DI water measured: _____ mL	Operator/Date	Verifier/Date
Sterilize filter solution using sterile 0.2 µm syringe filter as per the SOP: <u>Filter information:</u> Manufacturer: _____ Catalog number: _____ Lot number: _____	Operator/Date	Verifier/Date
Evaluate the integrity of the filter by performing the bubble point test per the Bubble Point Test SOP. Pressure needed to generate a steady flow of bubbles: _____ psig Does the filter pass the test (pressure >45psig): YES/NO (circle one)	Operator/Date	Verifier/Date
Aseptically add approximately 1.5mL of sterile filtered ampicillin stock solution (6mg/mL) to shake flask containing 100mL culture medium (Media must be cool to the touch!), swirl to mix. Volume of Ampicillin added: _____ mL	Operator/Date	Verifier/Date
Aseptically add 15mL of sterile filtered ampicillin stock solution (6mg/mL) to the 2L flask containing 1L agar medium, swirl to mix. Volume of Ampicillin added: _____ mL	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

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4. Pour Culture Plates/Proof LB Broth		
Pour agar plates and allow agar to solidify undisturbed with covers on as per SOP.	Operator/Date	Verifier/Date
Check for contamination. Autoclave and discard any plates showing microbial growth. Total number of plates: _____ Number of plates with contamination: _____	Operator/Date	Verifier/Date
Place the shake flask into the flask holders in the shaking incubator and incubate at 37 °C and 200rpm. Incubator ID: _____ Temperature: _____ °C Shaking Speed: _____ RPM Incubation Time: _____ hours	Operator/Date	Verifier/Date
Check for contamination. If contaminated, autoclave and dispose down the drain. Contamination: yes / no (circle one)	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

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5. Inoculation		
Prewarm shake flask containing 100ml LB/AMP/ARA medium at 37 °C.	Operator/Date	Verifier/Date
Remove the <i>E.coli</i> vial from storage in the -86 °C freezer. Vial ID: _____	Operator/Date	Verifier/Date
Thaw contents rapidly by agitation in a 37 °C water bath.	Operator/Date	Verifier/Date
Aseptically transfer the <i>E.coli</i> cells into the previously prepared shake flask per the SOP and immediately take a sample.	Operator/Date	Verifier/Date
Comments:	Operator/Date	Verifier/Date

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TIME POINT (min)	OD (550nm)	pH	Operator/Date	Verifier/Date
0				
60				
120				
180				
240				
300				
360				
420				
480				
540				
600				
660				
720				

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Time Point (min.)	Colony Count on 10 ³ plate	Colony Count on 10 ⁴ plate	Colony Count on 10 ⁵ plate	Colony Count on 10 ⁶ plate	Viable Cell Count (cells/ml)	Gram (-) Gram (+)	Glowing (Yes/No)	Operator/Date	Verifier/Date
0									
60									
120									
180									
240									
300									
360									
420									
480									
540									
600									
660									
720									

*** Attach growth curve and double time calculation.**