ScienceBridge Tech Site Standard Operating Procedure

Title: Pouring LB/Amp Agar Plates SOP		
Original Issue Date: 1/25/18	Revision Date: 5/1/2024	Pages: 3
Originally prepared by: Lindray Napalan and Tiffany Vilay	Revised by: Anthony Pham and Steven Pasag	Approved by: Kristina Mitchell

Scope	For use by biotechnology students at ScienceBridge Tech Sites when making LB amp plates for **Protein Purification** and **Bacterial Transformation Kits**.			
Objective	This SOP sets the procedural specifications for making Amp solutions which will later be added to LB solution bottles in order to pour LB/AMP plates.			
Consumable Materials (or ends up in kit)		1 Sleeve	1 Bottle	
	1. 1 sleeve 60 mm Petri dishes (20 plates) 2. 1ml clear microfuge tubes 3. 0.075 g of ampicillin (found in fridge inside a small, light green cylinder container that is labeled "AMP Microfuge Tubes") 4. Autoclaved LB Agar Bottle 5. Gloves 6. Lab tape (red)	1 1 1 1 3 inches	3-5 1	
Equipment	1. p1000 micropipette 2. Laminar Flow Hood or Other Sterile Counter 3. Fine Black sharpie. 4. 10% Bleach/ 70% isopropyl			
Supplemental Aids	• Teacher Spreadsheet • Preparing LB Agar SOP • QC & Packaging LB Agar Plates SOP • Site Prepping Autoclave for Solutions SOP • Media Batch Production Record • Making Solutions Tracking Sheet • Pouring LB / AMP Training Video			
Safety	• Use laminar flow hood or sterile countertop for sterile technique • Must wear gloves and use sterile technique by disinfecting workspace using 10% bleach/70% isopropyl alcohol with dH_2O • Clam shell plates when pouring to prevent plate contamination. • Wear gloves when holding the autoclave bottle. • **Be sure bottle is cool enough to hold before pouring plates**			
Quantity	• Plates needed per teacher: # of kits ordered x ½ sleeve + ½ sleeve • Calculate total plates needed for order date: add up all teachers • Needs to be recorded on spreadsheet for LB Plates			

	• 750 mL of LB is ~3-5 sleeves
Procedure	**Ampicillin (amp) solution should be prepared immediately before pouring LB/amp agar plates.** **LB/Amp Agar must be poured SAME DAY it is autoclaved and ampicillin is added following Pouring LB/Amp Agar Plates SOP.** **Part 1: Making Concentrated Ampicillin Solution** • **Follow the Making Concentrated Ampicillin Stock SOP** **Part 2: Adding Ampicillin solution** **1.** Wait for autoclaved LB agar to be cool enough to handle with gloved hands. **2. QC: Check that autoclaved LB agar bottle has cooled enough to hold with a gloved hand before pouring to ensure plates do not crack.** **3.** Using a p1000 micropipette, add the whole amp solution prepared in Part 1 into the autoclaved LB agar. **4.** Secure the lid onto the LB/amp bottle and gently swirl the bottle to fully mix. **5.** Be sure to label the bottle LB/AMP [Bottle Letter] [Date] [Initials] **Part 3: Pouring Plates** Use a red colored lab tape (or one that differs from the LB tape) to label all LB/AMP work spaces, bottles, and sleeves. 1. Sterilize laminar flow hood/ counter using 10% bleach or 70% isopropyl alcohol. If using a laminar flow hood, sterilize according to Laminar Flow Hood SOP. 2. Create about up to 3 sections (based on number of bottles made) in laminar flow hood/ on counter for using lab tape to demarcate each section. 3. Label each section with LB/AMP [Bottle Letter] [date] [initials]. 4. **QC: Check that each section is labeled to match label on bottle(s).** 5. Open plate sleeves by cutting a slit under the larger seal flap so that packaging later will be easier. 6. Spray gloved hands with 70% isopropyl alcohol to sanitize your hands before handling the plates 7. Remove plates and group in 3's to make counting and pouring easier. 8. Clamshell lids and carefully pour LB/AMP agar solution into plates until they are about $^{1}/_{3}$ to ½ full. a. Optional: pour solution into an autoclaved flask from the bottle for easier pouring. Follow the Autoclaving Glassware SOP to prepare flasks, if needed. 9. When finished with a stack of 3, slide plates aside (DO NOT PICK UP) so they can solidify. 10. Plates should be left at room temperature overnight. 11. Wash bottles and flasks in sink using regular faucet water.

	a. If agar solution has solidified in the bottle, use 10% soap solution and dH_2O and hang on drying rack to dry (MUST HAVE dH_2O FINAL RINSE)
Documentation	**All work and any variance from the protocol must be documented** • Always document in the communication logs • Bottles should be labeled with Solution Tracking # and bottle letter • Complete Making Solutions Tracking Sheet - & keep with bottle(s) • Complete Media Batch Production Record
Storage	**Always start with the oldest dates on supplies and complete partially finished or open bags first.** **Product Storage** • Plates stored in/on sterile laminar flow hood or counter at room temperature. **Supplies Storage Locations** • Ampicillin stock is found in fridge (in door) inside a small, light green cylinder container that is labeled "AMP Microfuge Tubes" • Plates are found in the Media Specialist Storage area, have to ask the instructor for site location.
Quality Control	•
Reagent Label Sticker	•
When	All plates must be finalized AT LEAST 1 WEEK BEFORE order date • Immediately complete calculations and Media Tracking Sheet • Determine how many plates to pour EACH WEEK to meet deadline Should always have a minimum of 4 sleeves in the fridge at all times.
Tech Site Kit: Group	Solutions Bacterial Transformation, Protein Purification