

ScienceBridge Tech Site Standard Operating Procedure

Title: Pouring LB Agar Plates SOP		
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Scope	For use by biotechnology students at a ScienceBridge Tech Site when LB agar plates are needed to maintain stock or complete kit orders.		
Objective	This SOP sets the procedural specifications for pouring LB plates.		
Consumable Materials (or ends up in kit)		1 sleeve	1 bottle
	1. 1 sleeve 60 mm Petri dishes (20 plates). 2. Autoclaved LB Agar Bottle 3. Lab tape (white) 4. Gloves.	1 3 inches 1 pair	3-5 1
Equipment	1. Laminar Flow Hood or Other Sterile Counter 2. Fine Black sharpie. 3. 10% Bleach/ 70% isopropyl	1 1 1	
Supplemental Aids	- Teacher Spreadsheet - Pouring LB Agar plates SOP - QC & Packaging LB Agar Plates SOP - Site Prepping Autoclave for Solutions SOP - Media Tracking Sheet - Making Solutions Tracking Sheet		
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 dH₂O - 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	LB Agar must be poured SAME DAY it is autoclaved following Pouring LB Agar Plates SOP. Pouring Plates 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 [Bottle Letter] [date] [initials]. 4. Open plate sleeves by cutting a slit under the larger seal flap so that packaging later will be easier. 5. Remove plates and group in 5's to make counting and pouring easier. 6. QC: Check that autoclaved LB agar bottle has cooled enough to hold with a gloved hand before pouring to ensure plates do not crack. 7. Clamshell lids and carefully pour LB agar solution into plates until they are about $\frac{1}{3}$ to $\frac{1}{2}$ 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. 8. When finished with a stack of 5, slide plates aside (DO NOT PICK UP) so they can solidify. 9. Plates should be left at room temperature overnight. 10. Wash bottles and flasks in sink using regular faucet water. If agar solution has solidified in the bottle, use 10% soap solution and dH₂O and hang on drying rack to dry (MUST HAVE dH₂O 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)
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 • 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 <u>BEFORE</u> 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 Group/ Kit	Media Specialist; Bacterial Transformation