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Preparation of Lipopolysaccharide for Intraperitoneal Injection

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the steps for preparing 20X stock and experimental solution of Lipopolysaccharide (LPS) intended for intraperitoneal injections in mice at a concentration of 0.25 mg/mL.

Guidelines

- LPS can be given at dose 0.1 to 2.5 mg/kg in saline via intraperitoneal injection at a max volume of 0.5 mL, for a maximum of 1 day.

Materials

	Material	Manufacturer/Supplier	Catalog Number
	5 mg LPS powder	Sigma-Aldrich	L3024-5MG
	Pharmaceutical grade sterile saline (0.9%)	Fisher Scientific	NC9054335
	20-200 µL Pipette	Rainin	17014391
	100-1000 µL Pipette	Rainin	17014382
	250 µL pipette tips	Mettler-Toledo	17014962
	1000 µL pipette tips	Mettler-Toledo	17014966
	1 mL syringe	Fisher Scientific	309659
	23-Gauge needle attachments	BD	BD305143
	Low protein binding tubes	Fisher Scientific	23-045-258
	Caps for low protein binding tubes	Fisher Scientific	22-038-265
	Ethanol swabs	Uline	13-680-63

⊗ Lipopolysaccharides from Escherichia coli O111:B4 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L3024-5mg**

⊗ 0.9% SODIUM CHLOR. INJ.10ML **Thermo Fisher Scientific Catalog #NC9054335**

⊗ Pipet-Lite LTS Pipette L-200XLS **Rainin Catalog #17014391**

⊗ LTS Pipette L-1000XLS **Mettler Toledo Catalog #17014382**

⊗ Pipette Tips TR LTS 250µL S 960A/10 **Mettler Toledo Catalog #17014962**

⊗ Pipette Tips TR LTS 1000µL S 768A/8 **Mettler Toledo Catalog #17014966**

⊗ 1 ml tuberculin syringes **Becton Dickinson (BD) Catalog #REF 309659**

⊗ BD® Needle 3/4 in. single use, sterile, 23 G **Becton Dickinson (BD) Catalog #305143**

⊗ Simport™ Scientific Microwtube™ Microcentrifuge Tubes with Low Adhesion Surface **Thermo Fisher Scientific Catalog #23-045-258**

⊗ Simport™ Scientific Nonsterile Screw Caps **Thermo Fisher Scientific Catalog #22-038-265**



Safety warnings

- Proper PPE includes lab coat, eye protection, and disposable gloves. Report any accidental sticks to EHS.
- When handling syringes with needles, always ensure needles are pointed away from you to avoid any accidental sticks.
- Used needles and syringes should be disposed of in biohazard sharps container.
- Lipopolysaccharide (LPS) is a potent immune stimulant and should be prepared in a fume hood and under sterile conditions with proper PPE.



Prepare 20X Stock Solution

1m

- 1 Upon arrival of  5 mg Lipopolysaccharide (LPS) powder, in a fume hood dissolve in  1 mL of pharmaceutical grade sterile saline (0.9%) to make a  5 mg/mL stock solution.

Note

Note: If stock solution isn't prepared upon arrival, store LPS powder at  4 °C .

- 1.1 Attach 23-Gauge needle to 1 mL syringe.
 - 1.2 Swab surface of sterile saline bottle septum with a 70% ethanol swab.
 - 1.3 Withdraw  1 mL volume.
 - 1.4 Eject saline in syringe into the bottle containing  5 mg LPS powder.
 - 1.5 Mix the contents for ~  00:01:00 by inverting and shaking the bottle to ensure all powder on the bottle's interior surface is dissolved.
- 2 Pipette  25 µL aliquots of stock solution in low protein binding tubes and store at  -20 °C .

1m



Note

Stock solutions can be stored at -20°C for approximately 2 years before expiring.

- 2.1 Remove label from the empty LPS bottle and discard bottle in biohazard waste.



2.2 Place label on clipboard that documents used chemicals in the lab.

Prepare 0.5 mg/mL LPS Working Solution for IP Injection

1m

3 At most  01:00:00 before the injection on the experimental day, dilute stock by 20X (to  0.25 mg/mL final concentration).

3.1 Rapidly thaw stock aliquot by holding fingers to the wall of the tube for about  00:01:00 .

1m

3.2 Add  475 μ L of saline and mix by inverting the tube or by vortexing.



4 Leftover LPS should be discarded in a biohazard waste container.