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Chronic LPS Paradigm

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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol describes the process to induce chronic systemic inflammation. It is composed of biweekly injections of sterile saline or 1×10^6 EU/Kg LPS for 6 weeks.

Guidelines

Prepare 1X LPS solution in a sterile biosafety or laminar flow hood and prepare 1X LPS solution fresh prior to giving injections.

Materials

LPS (*Escherichia Coli* 0111:B4, Sigma Aldrich, L2630)

Sterile Saline

10mL syringes (B302995, Thermo Fisher)

23 gauge needles (BD 305145, Thermo Fisher)

15mL tube

50mL tube

10mL Sterile, Empty, Clear Vial (18493, Greer)

no waste 1ml 25g 5/8" tuberculin syringes (14-841-34, Thermo Fisher)

Scale

Old tip container for weighing mice

Sharps container

Safety warnings

! Wear appropriate PPE and take caution with Sharps.

Before start

Day prior to first day of injections

1. Make sure all animals are appropriately separated by group, split cages if necessary.
2. Record the baseline weight of each animal and give each cage wet chow and a water bottle.

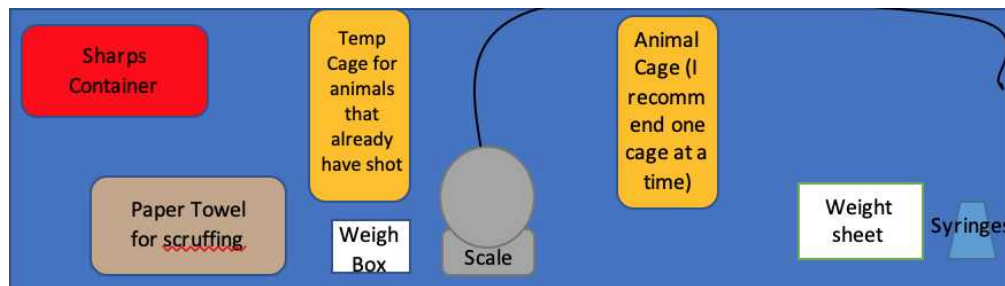
Preparing LPS injections

- 1 Prepare a 100X (10million EU/ML LPS) working stock of LPS
*Check your LPS lot to determine EU/ml if you have a less concentrated lot adjust your working stock concentrations but insure that your 1XLPS stock for injections is 1million EU/ml
- 2 Calculate how much 1XLPS stock you will need
 - a. $\text{Volume of 1XLPS} = (\text{Average weight of animals in grams}) * 10 * (\text{number of animals}) * 1.25$
 - b. Multiplying by 1.25 ensures that you have extra just in case
 - c. $\text{Volume of 100XLPS needed} = (\text{volume of 1XLPS}) / 100$
 - d. $\text{Volume of Saline needed} = (\text{volume of 1XLPS}) - (\text{volume of 100XLPS needed})$
- 3 Remove volume of saline needed plus a little extra from saline bag with 10ml syringe and 23g needle and transfer to 15mL tube
- 4 Transfer exactly volume of saline needed using a 1000uL pipette into the 50mL tube.
- 5 Vortex the 100X stock of LPS
- 6 Add exact volume of 100X stock needed into 50mL tube to bring up to volume.
- 7 Close the lid to the 50mL tube and vortex well
- 8 Using the other 10mL syringe and 23g needle transfer all of the volume of the 50mL tube into the 10mL vial
with rubber stopper this will make life much easier when drawing up LPS injections upstairs

Giving LPS injections

- 9 Bring 1X LPS (10ml vial with rubber stopper), the saline bag, a box of no waster insulin syringes with 25g needle pre-attached, a scale, an old tip container for weighing mice, a pencil, and the weight sheet up to the mouse room.
- 10 Once in the mouse room

- a. Put on appropriate PPE (I usually double glove-since the mice know you are there to give them a shot)
- b. Spray down the hood and set it up as shown below



- 11 Once setup is complete tare the scale with the weigh box on it.
- 12 Pick up one mouse from the cage by the base of its tail and identify it by your marking scheme and place the animal in weight box on the scale and record its weight in grams.
 - a. Note: Clean weigh box between cages, if an animal pees clean before next use, and after all shots are complete. Clean box with Peroxigard.
- 13 Then prepare the syringe of saline or LPS for that animal based on its treatment group. Injection volume is 10 times the animals weight in uL (i.e If an animals weighs 30g then the animals will need a 300uL injection)
- 14 Scruff the animal and give IP injection of appropriate treatment on the appropriate side (since animals receive biweekly shots, the side of injection switches every time)
- 15 Make sure to note if the shot goes subcutaneous at all, if there is a bleb, if there is blood, if there is sacring, anything notable about the shot or the animal.
- 16 Place the animal who has received the injection into the temporary cage and proceed so that all animals in the cage receive a shot.



- 17 Once all animals have received a shot place them back in their home cage and **give them a water bottle and wet chow** (be generous this is very important supportive care) and place them back in their rack.
- 18 For an efficient shot session I recommend one person identify animals, place one mouse at a time in the weigh box, and pull up the shot while the other person scruffs the mouse and gives the IP injection.
- 19 After the first and second shots, animals will need to be assessed that afternoon as to whether they need more supportive care. Animals will also need to be checked on each day after their first a second shots until their third round of shots to assess the need for extra supportive care. Extra supportive care comes in the form of subcutaneous saline shots up to 1ml/day (usually 500ul depending on how dehydrated they are).