LPS-induce acute lung injury (LPS nebulization, mice)

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Abstract

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Guidelines

Use PBS1X cold (4C) to ensure the correct performig of the bronchoalveolar lavage

Materials

- LPS (5mg/ml) L3129 by Sigma-aldrich
- 1 ml syringe 9161406V by Contributed by users
- Thread by Contributed by users
- NaCl 0.9% by Contributed by users
- PBS1X by Contributed by users

Protocol

Step 1.
Sacrifice the mouse with CO2

Step 2.
Isolate the trachea, making sure to clean the conjunctiva and surrounding fat well.

Step 3.
With the aid of a pair of tweezers, we pass the thread under the trachea, and make a pretense, without squeezing.

Step 4.
With the scissors make a small cut in the trachea, and put the tip of the needle with round tip.

Step 5.
Tighten the knot to make sure the needle does not come out of the trachea. We make a second knot tightening.

Step 6.

We do bronchoalveolar lavage by gradually introducing into the lungs 500ul of heparinized PBS 1X, we will see how the chest of the mouse swells.

Step 7.
Pick up washing slowly, it may help raise and tilt the mouse body slightly.to. It is normal to pick up only about 300-400ul of washing, partly because of the dead volume of the needle.

Step 8.
Next, we collect the lungs and rinse them in saline:to. We cut part of the lung and pick it up in the falcon with 10% formaldehyde to fix it. Subsequently we introduce a gauze to make sure the fabric is well embedded.b. The rest of the tissue is stored in the packets, frozen in liquid nitrogen and when we finish the experiment we store it at -80 ° C

Step 9.
Wash, centrifuge 10 min at 300G and 4 ° C. We resuspended the pellet in 0.5 ml and we count it in camera of Neubauer.

Warnings

Use mask and nebulize the solution of 5mg/ml of LPS in a laminar flow hood.