

Evaluation of lung permeability by FITC-Dextran intravenous injection

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Abstract

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Materials

FITC-Dextran 46944 by [Sigma Aldrich](#)

LPS L3129 by [Sigma-aldrich](#)

 Surgical matherial by Contributed by users

Protocol

Step 1.

1- We place the chamber connected to the nebulizer in a laminar flow hood

Step 2.

2- We load 10 ml of the 5 mg / ml dilution of LPS into the nebulizer vessel

Step 3.

3- We put in the nebulization chamber the mice

Step 4.

4- Turn on the nebulizer and wait 30 minutes, making sure the LPS is misting (we will see droplets condense into the tube coming out of the gas chamber).

Step 5.

5- We turned off the nebulizer and left the mice 10 minutes longer inside the chamber.

Step 6.

6- After 40 minutes, we took the mice out of the camera and took them to the cage. o We must be careful and throw the gloves with which we have touched the nebulization chamber and anything else that has been in contact with the LPS in a garbage bag that we will close before throwing. o Wash gas chamber and nebulizer to remove LPS debris.

Step 7.

7- After 24 hours, we continue: Dextran: - Anesthetize the mice with forane. - Inject 100µl of FITC-Dextran (25mg / ml) into the retroorbital sinus.

Step 8.

8- 30 minutes later, we sacrificed the mouse with CO2

Step 9.

9- We isolated the trachea, making sure to clean the conjunctiva and the surrounding fat well.

Step 10.

10- With the help of a pair of tweezers, we pass the thread under the trachea, and we make a bundle, without squeezing.

Step 11.

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

Step 12.

12- We tighten the knot to make sure the needle does not come out of the trachea. We make a second knot tightening.

Step 13.

13- 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 14.

14- We collect the washing slowly, it can help raise and tilt the mouse body slightly. o It is normal to pick up only about 300-400ul of washing, partly because of the dead volume of the needle.

Step 15.

15- Centrifuged for 10 min at 300G and 4 ° C - We performed a thoracotomy and cut the cava

Step 16.

16- We collect blood with a heparinized syringe, we reserve in ice.

Step 17.

17- We collected the supernatant and measured the fluorescence of the plasma (1:20 dilution) and of the BALF (100 µL) in a fluorimeter (CIC) with an excitation wave of 485 nm, and an emission wave of 528 nm.

Step 18.

18- Finally, we corrected the concentration of FITC-Dextran in BALF and lung by the concentration in blood: (FITC-Dextran Fluorescence in BALF) / (FITC-Dextran Fluorescence in Blood) (Fluorescence FITC-Dextran in homogenized lung) / (Fluorescence FITC-Dextran in blood)

Warnings

Use a laminar flow hood to nebulize the LPS.

Change your gloves after LPS procedure