

Thymectomy

Summary:

This protocol removes the thymus of the mouse to stop migration of newly matured cells to the periphery of the mouse.

Material

Anesthetic (Avertin)
Surgery plate with rubbers bands
Small surgery scissors
Two pairs of microdissecting curved forceps, tip width 0.8mm
Some suture string with curved needle (size 4-0, hooked 21 to 26 mm needle)
Michel wound clip and clip applying forceps
Warming plate or heating lamp

Avertin

Stock solution

5g tribromoethanol
5ml tert amylalcohol
Dissolve it carefully in water (need a thorough mixing. You can use 50ml tubes to be more comfortable to shake).

Aliquote in eppendorf tubes (1250 µl per aliquot), keep frozen and in the dark for up to one year.

Working solution

Dilute one tube of stock solution in 48.75 ml of PBS.
Store at +4 C in the dark for 6 months.
Shake well before each usage.

Dose

0.015 ml per g of body weight, injected i.p give around half an hour of sleep.
Use a lower dose for pups (.014 ml/g)
Use a higher dose for fat adults (.017 ml/g)
Try not to inject in the fat corpses, which will absorb partially the product, so that the dose in the body will be decreased.

References

2,2,2- tribromoethanol 99% Aldrich T48402
tert amyl alcohol 99+% Aldrich 240486

Technique

1. Anesthetize the mouse.
2. Check the reflex by pinching the finger. It shouldn't react any more when the mouse is asleep.
3. Flip the mouse on its back, maintained by some rubbers on the limbs and on the nose (hook it over the teeth). Check that the mouse's mouth is open so that it can breathe.
4. Wet the skin of the throat with ethanol 70 %. Pull the hair on both sides and make a vertical incision into the skin with scissors. The incision should be 2 centimeters, starting between the angle of the mandibul and following the sternum.
5. Stretch the borders with the forceps to see the thoracic cage about until the third rib.
6. Separate the submaxillary muscles until you see the trachea, being careful not to touch the vessels on both sides. If there is some blood, wait until it stops, it is not usually excessive.
7. Prepare the suture by passing it in 4 points, two through the submaxillary muscles and two in the muscles of the thoracic cage. Try to get as much empty space as possible around the top of the sternum.
8. Put the thumb and the index of your left hand on the bottom of the thoracic cage to maintain it, and with your right hand, insert one blade of small scissors under the sternum from the top. You should be careful that the tip of the blade is always as high as possible. Insert it approximately until the second rib,

and cut with a top-directed movement. The incision should be around 5 millimeters. As soon as the cage is open, you should finish within a few minutes, and let in as little air as possible.

9. With one pair of forceps, maintain the thoracic cage open, and use the other one closed to tear the membrane that is in front of the thymus.

10. Catch the thymus with the tweezers and bring it out of the thoracic cage by pulling it gently with one pair of forceps, and catching it a bit further with the next one several times. You should be able to get it in one piece. If it is not the case, quickly have a look in the thoracic cage to check that there is no piece left. As soon as the thymus is removed, close the hole by stretching the threads, and put the mouse back on its stomach very quickly, to help the mouse to breathe. Then make the knot, and close the skin with at least 2 clips.

11. Put the mouse in a clean cage and warm it. The best is to use a warming plate on thermostat 3 or 4 until the mouse starts crawling, which takes around half an hour. Check from time to time that the mouse is still breathing, you can have some mortality if too much air entered the thoracic cage.

Tips :

If you are not firm enough, the thymus will slip back deeper into the thoracic cage. But if you catch it too strongly, the thymus may be torn by the forceps, and it will be more tricky to be sure that you took everything out. If you catch the thymus from the bottom, you have a higher risk of touching the large vessels (lethal bleeding may then occur). The survival of the mouse is directly linked to the amount of air that can penetrate into the thoracic cage. You have to find an equilibrium between opening too much and let air enter or opening too little and having problems in getting the thymus out. I always get some mortality, especially when the thymus starts to break and that I want to catch all the pieces.

It is good at the end of the experiment, when you kill the mouse, to open the thoracic cage and check again if there is any thymus left. It happened very rarely to me.

Keywords : thymus, surgery, T lymphocytes