

Institute of Experimental Immunology / University of Zürich Viral Immunobiology / Prof. Dr. Christian Münz		
Date 04082016	Standard Operating Procedure <u>Type II pneumocyte isolation</u>	Pages 1/1

Injection anesthesia

1. Mice are weighted.
2. Mice are anesthetized by i.p. injection of 0.05 mg/kg Fentanyl, 5 mg/kg Midazolam, 0.5 mg/kg Medetomidin.
3. After 5 to 10 min, animals are tested for surgical tolerance by pinching the foot between two fingers, touching the eye or nose. Surgical tolerance is reached when there is no retraction reflex.

Euthanasia by exsanguination

1. The mouse is placed in supine position and secured to a dissection board with pins.
2. Disinfect mouse with 70% ethanol
3. Perform a length-wise cut through the skin from the abdomen up to the mouth.
4. Expose the abdomen by pulling the skin in opposite directions with forceps.
5. Open the peritoneal sac with a lengthwise cut and snip the inferior vena cava to exsanguinate.
6. Perfuse the lung with PBS.
7. Insert a catheter into the trachea and instill the lung with dispase followed by low-melting point agarose.
8. Let the agarose solidify and extract the entire lung.
9. Incubate with digestive enzymes and dissect into a single cell suspension.
10. Proceed with negative and positive magnetic selection to isolate type II pneumocytes.