

Oct 04, 2024

Sinai SCENT TMC - 5x5 Project Mouse Harvesting

DOI

<https://dx.doi.org/10.17504/protocols.io.dm6gpzp5dlzp/v1>

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Cellular Senescence Net...



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Protocol Citation: Sojin Kim 2024. Sinai SCENT TMC - 5x5 Project Mouse Harvesting. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.dm6gpzp5dlzp/v1>

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Protocol status: Working

We use this protocol and it's working

Created: October 04, 2024

Last Modified: October 04, 2024

Protocol Integer ID: 109185

Keywords: 5x5 project mouse harvesting, cellular senescence inducer intervention, cellular senescence, mice, sinai scent tmc, mouse

Abstract

We propose a total of 10 C57BL/6 mice (5 males and 5 females) to be studied for each study group or cellular senescence inducer intervention.

Materials

See the steps

Experimental Design

- 1 Males and females are grouped and housed in standard cages, provided LabDiet 5K0G and acidified water ad libitum. Euthanasia is performed by cervical dislocation (CMQ93-26).
 - **Control:** Tissues collected at 32 weeks.
 - **Doxorubicin:** IP injection (5 mg/kg) at 26 weeks on days 0 and 10, with tissue collection at 32 weeks.
 - **Palbociclib:** Daily oral gavage (150 mg/kg) for 10 days starting at 26 weeks, with tissue collection at 32 weeks.
 - **High-Fat Diet:** From 24 weeks, animals receive a 45% fat diet until tissue collection at 32 weeks.
 - **Aging Controls:** Tissues collected at 24 months.

Lung Tissue Collection

- 2 **Materials:** 26G needle, 1 mL syringe, balance, surgical board, 70% EtOH, scissors, 2 forceps, suture thread, 19G cannula, E-tube (1 per sample), normal formalin, tools for lung inflation, histology container with 10 mL of normal formalin (1 per sample)
- 3 **Preparation:** After euthanasia, apply 70% ethanol to the throat to wet the fur.
- 4 **Incision:** Lift the skin and make a vertical cut on the throat to expose the salivary glands. Use forceps to expose the trachea.
- 5 **Tracheal Access:** Make a horizontal incision between the second and third tracheal rings. Insert a 19G cannula and secure it with a suture thread.
- 6 *Optional: Go to "Bronchoalveolar Lavage Collection"*
- 7 *Optional: Go to "Blood Collection and Serum Separation"*
- 8 **Lung Isolation:** Carefully remove the lung with the trachea and cannula intact.



9 Tissue Processing:

- **Right Lobe:** Isolate, freeze in liquid nitrogen, and store at -80°C .
- **Left Lobe:** Fix in 10% formalin overnight, transfer to 70% ethanol, and prepare for histology.

Bronchoalveolar Lavage (BAL) Collection

- 10 **Materials:** 26G-syringe, 70% ethanol spray, surgery board with needles/pins, 2 forceps, 1 scissors, 4.0-suture string, 19G-cannula (brown color), PBS, 1 mL syringe, e-tube, notebook/paper, pens
- 11 Slowly inject 0.8 mL of ice-cold PBS into the lungs (speed: 0.1 mL/sec), pause for 2 – 3 sec, and then slowly retrieve the BAL. Record the collected volume of BAL and transfer BAL to the E-tube (label: BAL cells) on ice. Repeat Steps with another 0.8 mL of ice-cold PBS. Save the BAL in one tube/sample.
- 12 Save the collected BAL on the ice during harvest and then continue for the “BALF and BAL cell separation” procedure

BAL Fluid (BALF) and BAL cell separation

- 13 **Materials:** 1 mL pipette, tips, e-tubes, marker pen, dry ice, waste bin, e-tube rack, timer, PBS (keep on ice), ice bucket, RBC lysis buffer
- 14 Centrifuge BAL sample: 3,000 rpm, 10 min, 4°C
- 15 Transfer the supernatant (=BALF) to a new E-tube (label: BALF) → dry ice or liquid nitrogen → -80°C
- 16 **CRITICAL:** Cell pellet + 1 mL room temperature RBC lysis buffer → immediately vortex for 3 seconds →
Incubation: 5 min, ice. Incubation time is critical.
- 17 Centrifuge: 3,000 rpm, 10 min, 4°C
- 18 Discard supernatant using a pipette. Do not disturb the pellet.
- 19 If you want to count the WBC, proceed to step 5. Or you can save BAL cell → dry ice or liquid nitrogen →



-80°C

- 20 Optional: Cell pellet + 500 uL of ice-cold PBS. Make the single-cell suspension by pipetting. CRITICAL Un-even cell suspension affects cell number as well as the quality of the Cytospin

Blood Collection and Serum Separation

- 21 **Materials:** 26G-syringe, surgery board with needles/pins, 1 forceps, 1 scissors, 23G-syringe, e-tube
- 22 Open the abdominal cavity. Remove all abdominal organs to reveal the inferior vena cava, which is located in the middle of the back of the body.
- 23 Insert a 26G syringe into the inferior vena cava and collect the blood slowly.
- 24 Transfer the collected blood to the e-tube. CRITICAL do not drop the blood. gently transfer the blood from the syringe to E-tube unless RBC is destroyed and affects the colorimetric analysis.
- 25 Leave the tube at room temperature for 30 min. Do not close the lid
- 26 Close the lid and centrifuge: 1,000 g, 10 min, 4°C
- 27 Collect the supernatant (=serum) carefully and transfer it to a new e-tube. Saved it at -80°C