

# Adult mouse lung cell dissociation (on ice)

#### **Andrew Potter**

#### **Abstract**

This protocol was used to dissociate adult (8-10 wk) mouse lung tissue. The entire procedure is carried out on ice (to reduce artifact gene expression changes) and takes about half an hour. The yield was 16,240 non-RBC/mg tissue with 87% viability.

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#### **Guidelines**

## **Enzyme Mixes**

## Coll. A/Elastase/Dispase Enzyme Mix (1.5 mL)

90  $\mu$ L Collagenase A 100 mg/mL – 6 mg/mL final (Sigma, 10103578001) 150  $\mu$ L elastase 43 u/mL - 4.3 u/mL final (Worthington, LS002292) 150  $\mu$ L Dispase 90 u/mL – 9 u/mL final (Worthington, LS02100) 7.5  $\mu$ L 1 M CaCl2 – 5 mM final 7.5  $\mu$ L DNAse (125 U/mL) 1095  $\mu$ L PBS

--> save 0.5 mL of coll./elastase/dispase mix in separate 1.5 mL tube.

# B. Lich enzyme mix (1 mL)

899  $\mu$ L DPBS (no Ca, no Mg) - Thermo Fisher, 14190144 1  $\mu$ L 0.5 M EDTA - 0.5 mM final conc. 100  $\mu$ L Bacillus Licheniformis 100 mg/mL - 10 mg/mL final conc. - Sigma, P5380

#### **Before start**

- -Prepare enzyme mixes and leave on ice.
- -Cool centrifuges to 4 °C.

#### **Materials**

RBC Lysis Buffer R7757 by Sigma

DNAse A3778 by AppliChem

BSA by Contributed by users

#### **Protocol**

## Step 1.

Mince lung tissue on petri dish on ice for 2 min until fine paste.

# Step 2.

Weigh out 25 mg of tissue on petri dish. Using a sterile razor blade or forceps place 25 mg tissue in 1 mL lenzyme mix in 1.5 mL eppendorf tube, incubating on ice.



25 mg Additional info: minced lung tissue

#### Step 3.

Incubate on ice. Shake tube every 30 secs. Begin triturating at 2 mins. Triturate 10X every 1.5 minute (first w/tip cut).

## Step 4.

After 5 min pipet tissue + enzyme mix into petri dish on ice. Mince 2 min using razor blade to further break up residual chunks of tissue.

## Step 5.

Pipet tissue + enzyme back into 1.5 mL tube. Rinse petri dish with 0.5 mL coll. A/elastase/dispase enzyme mix and pipet into same tube.

# **■** AMOUNT

0.5 ml Additional info:

rinse petri dish

# Step 6.

Continue triturating on ice for 2 additional minutes until you reach 9 minutes total digestion time

## Step 7.

At 9 min total digest time let tube settle for one min on ice. The chunks of tissue should settle to the bottom of the tube, leaving released cells in the supernatant. Pipet 80% of supernatant onto 70  $\mu$ M filter on sterile 50 mL conical.

## Step 8.

Rinse filter w/6 mL ice-cold PBS/BSA 0.04%. Leave filter on 50 mL conical for next steps.

AMOUNT

6 ml Additional info: icecold PBS/BSA 0.04%

# Step 9.

Add additional 1 mL of 10 mg/mL b. lich enzyme mix to residual clumps of tissue in enzyme in the 1.5 mL tube.

**AMOUNT** 

1 ml Additional info: B. lich

enzyme mix

# Step 10.

Continue triturating on ice 10x every 1.5 minute for 10 additional minutes (20 min total time). Shake every 30 sec.

## **Step 11.**

Pipet entire volume to same 70  $\mu$ M filter - rinse w/7 mL ice-cold PBS/BSA 0.04%. Transfer flow-through to 15 mL conical.



7 ml Additional info: icecold PBS/BSA 0.04%

## **Step 12.**

Spin 300 g for five minutes at 4  $^{\circ}$ C. Remove all but 100  $\mu$ L of supernatant - being careful not to disturb pellet.

**■** TEMPERATURE

4 °C Additional info: spin

at 300 g

# **Step 13.**

Add 900  $\mu L$  RBC lysis buffer to pellet. Triturate 20X using 1 mL pipet set to 700  $\mu L$  and incubate for two min on ice.

AMOUNT

900 µl Additional info: RBC

lysis buffer

# **Step 14.**

Add 12 mL ice-cold PBS/BSA 0.04% to 15 mL conical to dilute RBC lysis buffer.

**■** AMOUNT

12 ml Additional info: ice-cold PBS/BSA 0.04%

## Step 15.

Spin 15 mL conical 120 g for ten min at 4  $^{\circ}$ C to pellet cells and remove platelets (platelets should remain in the supernatant).

**▮** TEMPERATURE

4 °C Additional info:

# **Step 16.**

Remove supernatant and re-suspend in 700  $\mu$ L ice-cold PBS/BSA 0.04%. Examine using hemocytometer w/trypan blue. Adjust concentration to 1000 cells /  $\mu$ L for 10X Chromium or 100 cells /  $\mu$ L for DropSeq.



 $700~\mu l$  Additional info: ice-cold PBS-BSA 0.04%