Thymus dissociation Version 2

Andrew Potter

Abstract
Protocol for thymus dissociation (10-week old CD-1 female).

Citation: Andrew Potter Thymus dissociation. **protocols.io**
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Guidelines

***Bacillus Licheniformis* enzyme mix (1 mg/mL enzyme):**
492 µL DPBS (No added Ca, Mg)
0.5 mM EDTA (0.5 µL of 0.5 M EDTA/mL)
125 U DNAse 1 / mL (2.5 µL)
5 µL of 100 mg/mL enzyme (final conc. 1 mg/mL)

***Bacillus Licheniformis* enzyme mix (2 mg/mL enzyme):**
487 µL DPBS (No added Ca, Mg)
0.5 mM EDTA (0.5 µL of 0.5 M EDTA/mL)
125 U DNAse 1 / mL (2.5 µL)
10 µL of 100 mg/mL enzyme (final conc. 2 mg/mL)

+12.5 mg of tissue

Materials

✓ Please see Guidelines for required materials. by Contributed by users

Protocol

Step 1.
Quickly isolate thymus and immerse in ice-cold PBS.

Step 2.
Place thymus on petri dish on ice using sterile forceps.

Step 3.
Remove red regions rich in red blood cells using razorblade.

Step 4.

Using razor blade, mince whole thymus on petri dish, on ice 2 min until fine paste.

Step 5.
Weigh out 12.5 mg tissue on petri dish.

Step 6.
Using razor blade, place tissue in 1.5 mL tube containing 0.5 mL digest mix (1 mg/mL) on ice.

AMOUNT

0.5 ml Additional info:
Digest mix (1 mg/mL)

Step 7.
Shake every 30 seconds to re-suspend tissue for 2 minutes.

Step 8.
At 2 min, triturate gently 10X using 1 mL pipet set to 400 µL.

Step 9.
For 3 additional minutes (5 min total time), every minute remove tube and triturate gently 10X using 1 mL pipet set to 400 µL.

Step 10.
Let tissue chunks settle for 1 min on ice.

Step 11.
At 6 mins total time, remove 80% (400 µL) of supernatant consisting of dissociated cells (leaving undissociated tissue chunks at the bottom of the tube) and apply to 30 µM filter on sterile 50 mL conical-rinse filter with 6 mL ice-cold PBS/BSA 0.04%. Save 50 mL conical and filter for next steps.

AMOUNT

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

Step 12.
Add additional 0.5 mL enzyme mix (2 mg/mL) to residual tissue chunks in 1.5 mL tube.

AMOUNT

0.5 ml Additional info:
Enzyme mix (2 mg/mL)

Step 13.
For 6 additional min (12 min. total), continue triturating gently (10x) every minute on ice.

Step 14.
After 12 min. total digest time, triturate digest mix 10X and transfer to 30 µM filter (the same tube/filter as

used previously).

Step 15.
Rinse filter with 6 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Step 16.
Transfer flow-through to 15 mL conical and spin down 650 G for 5 minutes at 4° C.

TEMPERATURE

4 °C Additional info: Spin down

Step 17.
Remove supernatant and re-suspend in 1 mL total volume PBS/BSA 0.04% in a 1.5 mL tube.

AMOUNT

1 ml Additional info: PBS/BSA 0.04%

Step 18.
Spin 610 G for 5 minutes at 4 °C.

TEMPERATURE

4 °C Additional info:

Step 19.
Remove supernatant and re-suspend in 1 mL ice-cold PBS/BSA 0.04%.

AMOUNT

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

Step 20.
Determine cell yield and viability using hemocytometer with trypan blue. Adjust concentration to 1,000 cells / µL for 10x Chromium or 100 cells / µL for DropSeq.