Thymus dissociation

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 transfer in ice-cold PBS.

Step 2.

Place thymus on petri dish on ice.

Step 3.

Remove red regions rich in red blood cells using razorblade

Step 4.

Mince whole thymus on petri dish, on ice (2 min) until fine paste.

Step 5.

Weigh out 12.5 mg tissue.

Step 6.

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.

Triturate gently 10X using 1 mL pipet set to 400 µL.

Step 9.

For a total of 5 mins, every minute subsequently, 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, remove 80% of supernatant (400 µL) and filter using 30 µM filter on 50 mL conical- rinse with 6 mL PBS/BSA 0.02%. Save 50 mL conical and filter for next steps.

AMOUNT
6 ml Additional info: PBS/BSA 0.02%

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 12 min, continue triturating gently (10x) every minute on ice.

Step 14.

Remove tube, triturate 10X and transfer to 30 µM filter.

Step 15.

Rinse with 6 mL PBS/BSA 0.02%.

AMOUNT
6 ml Additional info: PBS/BSA 0.02%

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 0.02% in a 1.5 mL tube.

AMOUNT
1 ml Additional info: PBS/BSA 0.02%

Step 18.

Spin 610 G for 5 minutes.

Step 19.

Remove supernatant and re-suspend in 1 mL PBS/BSA 0.02%.

AMOUNT
1 ml Additional info: PBS/BSA 0.02%

Step 20.
Examine using hemocytometer with trypan blue.