VERSION 3

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 We use this protocol and it's working

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Adult mouse thymus dissociation (on ice) V.3

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ABSTRACT

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

ATTACHMENTS

[thymus_final_1_31_18.pdf](#)

GUIDELINES

Storage Conditions of Reagents

Reagent	Storage Condition

DPBS (Thermofisher, 14190144)	4 °C
0.5 M EDTA (Ambion, AM9260G)	room temp.
BSA (Sigma, A8806)	4 °C

P ro te a s e fr o m <i>B a c i l l u s L i c h e n i fo r m is</i> (<i>S i g m a</i> , p 5 3 8 0)	S to re 1 0 0 μ L al i q u o t s (1 0 0 m g / m L) in D P B S at - 8 0 ° C
D N A s e 1 (<i>A p p l i c h e m</i> , A 3 7 7 8)	S to re 1 0 0 μ L al i q u o t s (2 5 0 U / 1 0 μ L) in D P B S at - 8 0 ° C

***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

MATERIALS

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

- 1 Quickly isolate thymus and immerse in ice-cold PBS.
- 2 Place thymus on petri dish on ice using sterile forceps.
- 3 Remove red regions rich in red blood cells using razorblade.
- 4 Using razor blade, mince whole thymus on petri dish, on ice 2 min until fine paste.

00:02:00 Mincing
- 5 Weigh out 12.5 mg tissue on petri dish.

- 6 Using razor blade, place tissue in 1.5 mL tube containing 0.5 mL digest mix (1 mg/mL) on ice.
0.5 mL Digest mix (1 mg/mL)
- 7 Shake every 30 seconds to re-suspend tissue for 2 minutes.
00:02:00 Re-suspending
00:00:30 Shake
- 8 At 2 min, triturate gently 10X using 1 mL pipet set to 400 μ L.
- 9 For 3 additional minutes (5 min total time), every minute remove tube and triturate gently 10X using 1 mL pipet set to 400 μ L.
00:03:00 Digest on ice
00:01:00 Remove tube and triturate gently 10X
- 10 Let tissue chunks settle for 1 min on ice.
00:01:00 On ice
- 11 At 6 mins total time, remove 80% (400 μ L) of supernatant consisting of dissociated cells (leaving undissociated tissue chunks at the bottom of the tube) and apply to 30 μ M 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.
6 mL ice-cold PBS/BSA 0.04%
- 12 Add additional 0.5 mL enzyme mix (2 mg/mL) to residual tissue chunks in 1.5 mL tube.
0.5 mL Enzyme mix (2 mg/mL)
- 13 For 6 additional min (12 min. total), continue triturating gently (10x) every minute on ice.

00:06:00 Digest

00:01:00 Triturate gently (10x)

- 14** After 12 min. total digest time, triturate digest mix 10X and transfer to 30 μ M filter (the same tube/filter as used previously).

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

6 mL ice-cold PBS/BSA 0.04%

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

4 °C Spin down

00:05:00 Spin down

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

1 mL PBS/BSA 0.04%

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

00:05:00 Spinning

4 °C

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

1 mL ice-cold PBS/BSA 0.04%

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