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Adult mouse thymus dissociation (on ice) V.3

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Human Cell Atlas Metho...

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Attachments

thymus_final_1_31_18...

50KB

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
	Protease from *Bacillus Licheniformis* (Sigma, P5380)	Store 100 µL aliquots (100 mg/mL) in DPBS at -80°C
	DNAse 1 (Applichem, A3778)	Store 10 µL aliquots (250 U/10 µL) in DPBS at -80°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.

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 µL.

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.
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 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.

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 µM 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 / µL for 10x Chromium or 100 cells / µL for DropSeq.