

Pancreas Cell Dissociation

7.31.18

Type 4 Collagenase Enzyme Mix (2 x 1 mL)

50 µL Type 4 Collagenase 100 mg/mL (5 mg/mL final conc.)

5 µL DNase (125 U)

5 µL 1 M CaCl₂ (5 mM final conc.)

10 µL 10% BSA/PBS (0.1% BSA final conc.)

930 µL DPBS

Reagents

Collagenase Type 4 - Worthington (LS004186) - 100 µL aliquots of 100 mg/mL - frozen at -80 °C

DNase 1 - Applichem (A3778) – 10 µL aliquots each with 250 U

DPBS - ThermoFisher (cat. #14190)

Red Blood Cell Lysis Buffer - Sigma (R7757)

Trypan Blue Solution 0.4% - Gibco (15250061)

Cell yield

4,400 cells/mg with 94% viability

Protocol

1. Dissect pancreas and place in ice-cold PBS
2. Mince tissue thoroughly on petri dish on ice (2 min) until fine paste.
3. Weigh out 18 mg tissue and add to tube with 1 mL Type 4 collagenase enzyme mix.
4. Incubate on ice. Shake vigorously every 30 seconds for the first two min to re-suspend tissue. At two mins, begin triturating 10x every min.
5. Continue triturating on ice for 20 min. At 20 min, let chunks settle for 1 min on ice.
6. Pipet top 75% (750 µL) of supernatant containing released cells onto a 30 µM filter on a 50 mL conical, on ice. Rinse filter with 5 mL ice-cold PBS/BSA 0.04%. Save filter and flow-through for next steps.
7. To residual tissue chunks add additional 1 mL type 4 collagenase enzyme mix.
8. Continue triturating on ice 10x every min for 30 additional min (50 min total digest time).
9. Triturate and add entire volume to same 30 µM filter on 50 mL conical. Rinse filter w/5 mL ice-cold PBS/BSA 0.04%.
10. Transfer flow-through to 15 mL conical. Spin 300 g for 5 min. at 4 °C.
11. Remove supernatant and re-suspend in 100 µL ice-cold PBS/BSA 0.04%.
12. Add 900 µL RBC lysis buffer. Triturate 20x and incubate 2 min. on ice.
13. Add 10 mL ice-cold PBS/BSA 0.04% to dilute RBC lysis buffer.

14. Spin 300 g for 5 min. Remove supernatant.
15. Re-suspend in 100 μ L ice-cold PBS/BSA 0.04%.