Protocol 303_Small intestine preps for FACS

Reagents

HBSS
Liberase C1 aliquots (Roche)
40µl cell strainers
1ml syringe plungers

Protocol

1. Collect 2-3cm segments of small intestine. Remove any peyers patches using a scalpal blade
2. Cut the piece longitudinally to open the gut tube, then into 0.5cm across-ways (massively improves digestion)
3. Put into 1.5ml eppendorf containing HBSS (can contain BSA but doesn't make much difference)
4. Store on ice until all dissection is complete

Then...

5. Add Liberase C1 to a final dilution of 1:40 to each tube
6. Incubate at 37ºC in the shaking hot-blocks, shaking at about 800rpm for exactly 25mins (NOT LONGER – all cells will die!)
7. Return tubes to ice
8. Mash tube content through a 40µl cell strainer into a 50ml Falcon tube
9. Rinse through cell debris with 5ml HBSS (ice-cold – quite a lot of material will remain)
10. If material that has passed through the filter appears clumpy, pipette with a 1ml gilson-type pipette to disrupt
11. Centrifuge at 1500rpm for 5mins
12. Discard supernatant

Resuspend in buffer of choice for FACS etc