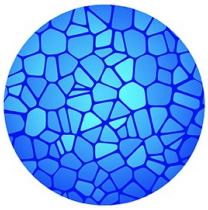
Aug 30, 2019

Tissue dissociation protocol for Pan Immune Project Tissue

DOI
https://dx.doi.org/10.17504/protocols.io.ymzfu76



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

Created: February 27, 2019

Last Modified: August 30, 2019

Protocol Integer ID: 20889

Keywords: pan immune project tissue, tissue

Attachments

DOCX

Tissue dissociation ...

13KB

1 Chop tissues into small pieces, weigh

2 Add <5.0g per miltenyi C tube with 5mL 1x liberase/DNase in xvivo15 + 1%FCS

3 Run on GentleMACS Octo 30 mins/37oC cycle

4 Stop liberase with 2mM EDTA

5 Mash through smart strainer

6 PBS wash/spin cells to pellet at 500g/10 minutes. Resuspend pellet in PBS.

7 Layer onto ficoll and spin at RT 400g/25 minutes

8 Remove MNCs from ficoll later and wash with PBS

9 Filter through 0.2uM filter

10 Count viable cells and resuspend at concentration of 1000/uL in 10X buffer (chilled PBS + 0.05% FBS) for loading to 10X