Standard operating procedure for single cell isolation from fresh bovine spleen tissue

modified after the protocol from Cheng H-W et al. (2019). (PMID: 30988302)

Research Institute for Farm Animal Biology (FBN)

Institute of Genome Biology, Genome Physiology Unit

Written by Doreen Becker (becker.doreen@fbn-dummerstorf.de)

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Purpose

Collecting single cells from fresh bovine spleen tissue. Appropriate preparation of required materials and professional training of lab technicians involved cell isolation have to be performed in advance.

Materials

- pipettes, pipette tips
- 1X PBS
- beakers
- centrifuge, table centrifuge
- RPMI1640 + 2% FBS + 20 mM HEPES + 0.2 mg/ml Collagenase P + 0.8 U/ml Dispase I + 100 µg/ml DNase I
- 50 ml Falcon tubes
- heater shaker
- MACS buffer (1X PBS, 1% FBS, 10 mM EDTA)
- Red Blood Cell lysis buffer
- AO/PI
- Cellometer Auto2000

Single-cell isolation

1. the animals are killed according to regular slaughtering protocol including stunning and exsanguination by expert staff in an experimental slaughterhouse of the institute

2. spleen tissue is collected during slaughter by expert staff and distributed to trained lab technicians for further sample preparation

3. spleen tissue is washed with cold 1X PBS

4. walnut-sized piece of spleen is cut from the tissue (without capsule) and placed in a beaker with cold 1X PBS (for transport to the laboratory)

5. tissue chopped into small pieces (1-2mm2)

6. tissue pieces are incubated in a 50 ml Falcon with RPMI1640 medium, 2% FBS, 20 mM HEPES, 0.2 mg/ml Collagenase P, 0.8 U/ml Dispase I and 100 µg/ml DNase I for 30 min at 37°C and gentle agitation (200 rpm) (amount of medium depends on the number of tissue pieces, the pieces should be well covered by the medium)

7. after 15 min the supernatant is collected in MACS buffer (1X PBS, 1% FBS, 10 mM EDTA)

8. cell suspension is centrifuged with 400 g for 7 min at 4°C and the supernatant is discarded

9. cells are resuspended in 20 ml cold (1X) Red Blood Cell lysis solution and the Falcon is inverted several times

10. cells are incubated in 1X Red Blood Cell lysis solution for 5 min at room temperature

11. Falcon is centrifuged with 400 g for 7 min at 4°C and the supernatant is removed

12. cells are resuspended in 20 ml MACS buffer

13. cells are centrifuged with 400 g for 7 min at 4°C and the supernatant is removed

14. cells are resuspended in 10 ml 1X PBS + 0.04% BSA

15. cell concentration and viability are measured using AO/PI staining and a Cellometer Auto2000 (Nexcelom)