



Jul 23, 2021

HPAP Processing Protocol

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1 Works for me



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dx.doi.org/10.17504/protocols.io.bvp5n5q6

Human Islet Research Network



Lili Liang

ABSTRACT

Early in life, a combination of environmental insults and genetic pre-disposition results in an autoimmune reaction to pancreatic b-cells in the Islets of Langerhans, leading to b-cell destruction and a lack of insulin production. Over time, near-complete loss of insulin production leads to Type I Diabetes (T1D), an often-deadly disease that requires lifetime treatment. Many studies have focused on immune system components that drive T1D autoimmunity, particularly the immune cells that infiltrate the islets of Langerhans. However, acute insulinitis is extremely difficult to detect, as T1D pancreas samples are rare and individual islet destruction is thought to happen rapidly and sporadically. Therefore, studies have focused on autoimmune imprints within memory T and B cells that may develop after insulinitis. Memory T and B cells that target b-cells would likely reside in lymph nodes that drain the pancreas and are potentially detectable in circulation or in the spleen. The Human Pancreas Analysis Program has organized a protocol to isolate single immune cells from the blood, spleen, and peri-pancreatic lymph nodes (PLNs) from healthy, pre-diabetic, and T1D patients. Herein, we describe the standardized protocol of single cell isolation from blood, spleen, PLNs, mesenteric LNs, and superior mesenteric LNs.

DOI

dx.doi.org/10.17504/protocols.io.bvp5n5q6

PROTOCOL CITATION

Michael Betts, Gregory Golden 2021. HPAP Processing Protocol. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bvp5n5q6>



KEYWORDS

null, T1D autoimmunity, HPAP, HIRN

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CREATED

Jun 10, 2021

LAST MODIFIED

Jul 23, 2021

PROTOCOL INTEGER ID

50653

MATERIALS TEXT

- [ACK Lysing Buffer 100mL Quality](#)
- **Biological Catalog #118-156-721**
- Collagenase-D, 10mg/mL
- [Dimethyl sulfoxide 100mL Sigma](#)
- **Aldrich Catalog #D2650-100ML**
- [DNase I Recombinant, RNase free \(10000U/mL\) Sigma](#)
- **Aldrich Catalog #4716728001**
- [Fetal Bovine Serum 500mL Gemini](#)
- **Bioproducts Catalog #100-500**
- [Ficoll Paque Plus 500mL Ge](#)
- **Healthcare Catalog #17-1440-03**
- [L-glutamine 200mM 100mL](#)
- **Corning Catalog #25-005-CI**
- [Penicillin/streptomycin 10000 U each 100mL](#)
- **Lonza Catalog #DE17-602E**
- PFA: 4% in PBS
- [RPMI 1640, 1x with L-glutamine, 1L Corning Catalog #10-040-CM](#)
- Conical, 50mL
- [Cryovials](#)
- [2mL Corning Catalog #430488](#)
- Freezing container, Corning CoolCell
- Sterile Forceps
- [gentleMACS C Tubes Miltenyi](#)
- **Biotec Catalog #130-093-237**
- [gentleMACS Dissociator Miltenyi](#)
- **Biotec Catalog #130-093-235**
- [MACS SmartStrainers 70µm Miltenyi](#)
- **Biotec Catalog #130-098-462**
- [MACS SmartStrainers 100µm Miltenyi](#)
- **Biotec Catalog #130-098-463**
- [SepMate-50 Stemcell](#)
- **Technologies Catalog #85450**
- Sterile Scalpel
- Sterile Syringe, 5mL

1 BLOOD

1. Spin down vials at 2000rpm for 15min at room temperature
2. Aliquot plasma into cryovials and freeze and store at -80°C ; toss remaining plasma
3. Measure volume of spun down blood and transfer to a new conical tube

4. Using a 1:1 ratio of spun blood to R10 media, wash vials and transfer to the conical with the spun down blood
 - a. R10 media = RPMI + 10% fetal bovine serum + 2mM L-Glutamine + 100 U/mL Penicillin + 100 µg/mL Streptomycin
5. In a SepMate™-50 conical, add **15 mL** of ficoll below the physical barrier
6. Slowly add the blood to the top of the ficoll, taking care not to mix the two
7. Spin down at **1200 g** for 10min at room temperature
8. Once separated, pour the supernatant with the PBMCs into a fresh conical
9. Spin down at **500 g** for 7min
10. Pour off supernatant and resuspend the pellet in ~2mL ACK Lysis buffer
11. Incubate at room temperature for 5min and then quench with **18 mL** R10 media
12. Spin down at **500 g** for 7min
13. Resuspend pellet in **10 mL** of media
14. Count cells
15. Spin down at **500 g** for 7min
16. Pour off supernatant and resuspend the pellet in FBS + 10% DMSO at 5-10 million cells per mL
17. Freeze in **1 mL** aliquots at **-80 °C** in a Mr. Frosty freezing container for 24-48hrs
18. For long term storage, store in liquid nitrogen



2 LYMPH NODE PROCESSING

1. Prepare media by adding **50 µl** DNase + **50 mL** R10 media into one conical for each tissue type
2. Weigh tissue in a culture dish and record mass
3. Add **10 mL** of prepared media to the culture dish
4. Place tissue in dish and gently cut away the fat
5. Rinse with **10 mL** of prepared media
6. For histology:
 - a. Cut off a small piece of tissue (~1-2cm) and place in **50 mL** freshly prepared PBS + 4% PFA solution
 - b. Write date and time on the tube and store for ~24hrs at RT
 - c. Transfer to **50 mL** conical with 80% EtOH and store at **4 °C**
7. Place tissue pieces in a 70µm cell strainer and grind the tissue with the top end of a **5 mL** syringe
8. Strain media in plate through the cell strainer into a fresh **50 mL** conical

9. Rinse plate several times with remaining media and strain through the cell strainer into the conical
10. Spin down at **500 g** for 7min
11. Resuspend pellet in **10 mL** of R10 media
12. Count cells
13. Spin down at **500 g** for 7min
14. Pour off supernatant and resuspend the pellet in FBS + 10% DMSO at 5-10 million cells per mL
15. Freeze in **1 mL** aliquots at **-80 °C** in a Mr. Frosty freezing container for 24-48hrs
16. For long term storage, store in liquid nitrogen

3 SPLEEN PROCESSING

1. Prepare media by adding **50 µl** DNase + **50 µl** Collagenase + **50 mL** R10 media
2. Weigh tissue in a culture dish and record mass
3. Cut tissue into ~2cm pieces
4. Dissociate using a gentleMACS with the tissue suspended in prepared media
5. Incubate for 15min at **37 °C** on a rotator
6. Repeat gentleMACS dissociation
7. Strain suspension through a 100µM filter
8. Spin down at **500 g** for 10min
9. Pour off supernatant and mix in ~10mL ACK Lysis buffer
10. Let sit for 5min and then quench with **40 mL** of R10 media
11. Strain suspension through a 70µM filter
12. Spin down vials at **500 g** for 10min at room temperature
13. Add **10 mL** ficoll to a clean **50 mL** conical
14. Resuspend cells and very carefully add to the top of the ficoll
15. Spin down at 2200rpm for 20min with the brake off
16. Resuspend cells with **10 mL** of R10 media
17. Dilute **10 µl** of the suspension in **990 µl** of PBS

18. Count cells and multiply by 1000 to obtain the final cell count
19. Spin down at  500 g for 10min
20. Pour off supernatant and resuspend the pellet in 20-30mL FBS + 10% DMSO
21. Freeze in 20-30 aliquots at  -80 °C in a Mr. Frosty freezing container for 24-48hrs
22. For long term storage, store in liquid nitrogen