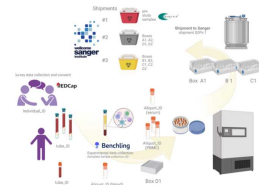


Jun 26, 2023

Blood Sample collection and PBMC isolation JAGUAR.v4

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvwjx4zlmk/v1>



Carla Jones¹, Gosia Trynka¹, Pablo Romagnoli²

¹Wellcome Sanger Institute; ²Instituto Universitario de Ciencias Biomédicas de Córdoba

Human Cell Atlas Metho...



Carla Jones

Wellcome Sanger Institute

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.e6nvwjx4zlmk/v1>

External link: https://www.sanger.ac.uk/news_item/researchers-will-map-immune-cells-across-latin-america-with-support-from-czi/

Protocol Citation: Carla Jones, Gosia Trynka, Pablo Romagnoli 2023. Blood Sample collection and PBMC isolation JAGUAR.v4. protocols.io <https://dx.doi.org/10.17504/protocols.io.e6nvwjx4zlmk/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: June 01, 2023

Last Modified: June 26, 2023

Protocol Integer ID: 82762

Keywords: blood for dna extraction, pbmc from healthy donor, jaguar sites for multimodal single cell analysis, dna extraction, blood sample collection, pbmc, dna, jaguar site, blood, multimodal single cell analysis, healthy donor

Funders Acknowledgements:

Chan Zuckerberg initiative

Grant ID: 2021-240438

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to [protocols.io](https://dx.doi.org/10.17504/protocols.io.e6nvwjx4zlmk/v1) is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with [protocols.io](https://dx.doi.org/10.17504/protocols.io.e6nvwjx4zlmk/v1), can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

AIM: This protocol aims to isolate and cryopreserve PBMC from healthy donors from JAGUAR sites for multimodal single cell analysis and blood for DNA extraction for genotyping.

Image Attribution

image created in Biorender



Materials

Reagents:

- RPMI 1640 (Gibco, cat#22400-089)
- Washing media (RPMI 1% serum, 1% Lglu Lonza, Cat. #BE17-605E/U1, 1%Pen/Strep ThermoFisher, Cat. #15140122)
- Isopropanol (if using Mr.Frosty, Sigma-Aldrich, cat n° C1562-1EA) or Coolcell
- Serum (FBS) Sigma, Cat#F7524-500ml
- 2X Freezer media (each 1mL contains 800 µL RPMI and 200 µL DMSO (20%))
- Cryovial (V7634)
- BD Vacutainer CPT (CPT tube) Cat# 362753
- DMSO (Sigma)

Equipment:

- Mr. Frosty or CoolCell (CLS432001-1EA)
- Centrifuge (refrigerated swinging bucket centrifuge, e.g. Beckman Coulter Allegra X-12R)
- Ultra freezer (-80 oC freezer)
- Liquid Nitrogen tank (LN2)
- Sterile Pasteur pipette (**E1414-0310**)
- 15 mL Falcon tube (CLS430052)
- 2D barcode labels (Ziath)

Before start

coolcell must be at room temperature



Blood Collection

15m

- 1 Collect blood (21g needle) into the tube using the standard technique for CPT tube (3 tubes per donor). After collection, store tubes upright at room temperature (RT, 18-25°C) for up to 2hr (if longer times are required keep tubes on ice until processing).

10m

Safety information

Work with human samples carries a risk of contamination. Needles and other sharps may expose workers to bloodborne pathogens. Consult healthy and safety personnel before using this protocol.

- 2 Collect an extra 1-2mL of blood in an EDTA blood collection tube and transfer blood to a cryotube labeled with a 2D barcode for DNA extraction (whole genome sequencing) and store at -80 until processing

5m

PBMC isolation

49m

- 3 After centrifugation, pipette to remove approximately half of the plasma (stored in a new tube at -80°C) without disturbing the cell layer. Collect the cell layer with a Pasteur Pipette and transfer it to a 15 mL Falcon tube with a cap. *Collection of cells immediately following centrifugation will yield the best results.
- 4 Centrifuge CPT tubes at 1500g RT for 30 minutes (break and acceleration at 5)
- 5 Add 2mL washing media to the CPT tube to wash remaining cells and transfer to the same tube above, bring volume to 10 mL. Cap tube. Mix cells by inverting the tube slowly 5 times.
- 6 Centrifuge for 5 min at 300 RCF. Tip off supernatant without disturbing the cell pellet.
- 7 Add washing media to bring volume to 10 mL. Cap tube. Resuspend cells gently with a pipette
- 8 Count cell numbers and viability using Trypan Blue (dilution 1:2) in a Neubauer chamber
CRITICAL: add 2D barcode stickers to the cryotubes while tube are at room temperature.

5m

30m



2m

5m



2m

5m



PBMC cryopreservation

15m

- 9 Centrifuge for 5 min at 300 RCF. Aspirate as much supernatant as possible without disturbing the cell pellet. Resuspend pellets in 500 µl of 100% FCS and transfer all to a cryovial. 5m 
- 10 Combine 500 µl 2X Freezer media (dropwise) with the cells in cryovial and gently pipette to mix. Place the cryovial into the Mr. Frosty (or CoolCell) and transfer to -80 °C as soon as possible. 5m
- 11 CRITICAL: cell viability will be directly correlated to the time cells spend in the presence of DMSO - the quicker the better 
- 12 After 24 hrs, move cryovials into liquid Nitrogen tanks until shipping, record date of liquid nitrogen transfer 5m 

Protocol references

Pradeep K. Dagur and J. Philip McCoy Jr (2015) Collection, Storage, and Preparation of Human Blood Cells. Curr Protoc Cytom. ; 73: 5.1.1–5.1.16. doi:10.1002/0471142956.cy0501s73

Puleo A, Carroll C, Maecker HT, Gupta R. Isolation of Peripheral Blood Mononuclear Cells Using Vacutainer® Cellular Preparation Tubes (CPT™). Bio Protoc. 2017 Jan 20;7(2):e2103. doi: 10.21769/BioProtoc.2103. PMID: 34458433; PMCID: PMC8376597.

BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube - Sodium Heparin