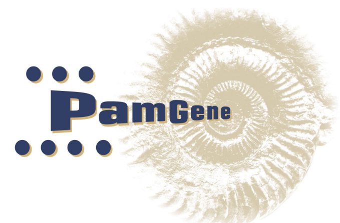
Protocol 1171

Sample preparation of Peripheral Blood Mononuclear Cells (PBMCs) for kinase and phosphatase activity profiling



Protocol for sample preparation of Peripheral Blood Mononuclear Cells (PBMCs) for shipment to PamGene
Version 4

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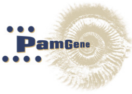

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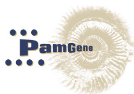


1 INTRODUCTION

1.1 Intended use

The aim of this protocol is the preparation of PBMC's for shipment to PamGene. These frozen cells will be thawed, washed once and lysed internally at PamGene according to protocol 1160, for preparation of lysates of purified cells" for analysis by PamChip® Kinase and/or Tyrosine Phosphatase activity profiling.

2 PROTOCOL

2.1 Materials & reagents

- Dulbecco's Phosphate-Buffered Saline (PBS), no calcium, no magnesium (GIBCO 14190094)
- Ficoll-Paque PLUS (GE Healthcare Life Sciences # 17-1440-03)
- RPMI 1640 Medium (GIBCO # 52400-025)
- Fetal Bovine Serum (FBS), heat inactivated (GIBCO # 10500-064)
- Pierce™ Dimethylsulfoxide (DMSO), LC-MS Grade (Thermo Scientific # 85190)
- RPMI-10: 90% RPMI 1640 Medium + 10% FBS
- Freezing medium: 20% DMSO + 80% FBS
- Cryovials (f.i. Nunc™ Biobanking and Cell Culture Cryogenic Tubes; Thermo Scientific # 374080)

2.2 Equipment

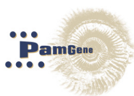
Container to safely use liquid nitrogen (or if liquid nitrogen is not available a -80°C freezer)

2.3 Safety & Precautions

Blood is a body fluid and should be considered biohazardous. Protective equipment should be worn at all times and blood should be disposed according to the regulations of the researcher's institution.

2.4 Cleaning Tools and Equipment

No special cleaning required other than specified in the regulations of your institution.



2.5 Procedure

10 ml blood yields about 0.5 - 2 x 10^7 PBMCs. To obtain higher numbers of PBMCs, several Ficoll isolations can be performed simultaneously.

1. Collect heparin blood into vacutainer tubes and keep at Room Temperature (RT).
2. Within 6 hrs, open tubes in a laminar flow hood and pipet the blood from the vacutainer tube into a 50 ml tube.
3. Rinse vacutainer tube with PBS and add to the blood in the 50 ml tube to obtain a 1:1 dilution of blood with PBS up to maximally 35 ml
4. Put the diluted blood on top of Ficoll via the wall of a new tube prefilled with 10 ml Ficoll.
5. Spin at RT at 1000 *x g* for 15 minutes, brake off (braking would result in mixing of layers).
6. The layers are from top to bottom: Plasma – platelets – PBMC – Ficoll – granulocytes – red blood cells (RBC).

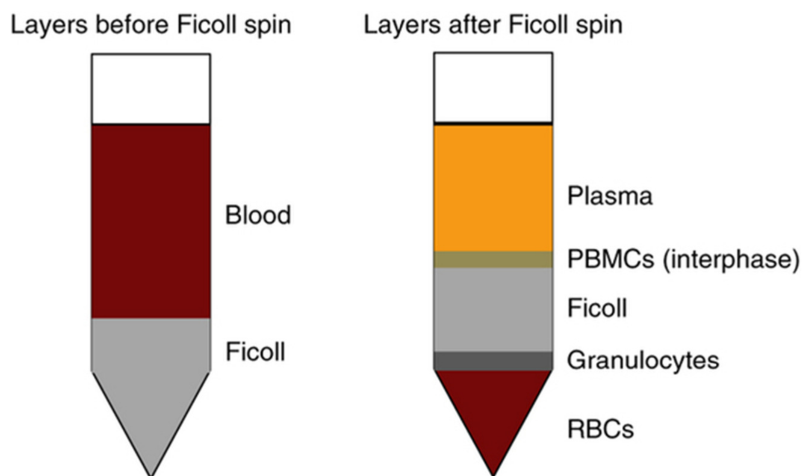
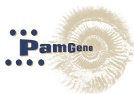


Fig. 1: The layers before and after Ficoll separation of blood.

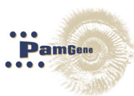
7. Pipet off as much plasma as possible, discard according to the protocol used in your institution or store at -20 to -80°C.
8. Carefully aspirate the interphase with PBMC's and transfer to a 50 ml tube. Inclusion of some plasma is no problem, but minimize the aspiration of Ficoll.
9. Discard the remainder of the Ficoll and red blood cells according to the protocol used in your institution.
10. Wash PBMCs with up to 50 ml PBS (RT).
11. Spin (RT) at 500 *x g* for 10 minutes, brake on (cell pelleting).



12. Resuspend **in 50 ml RPMI-10** and centrifuge for 5' at 500*g* (RT), brake on (cell pelleting).
13. Decant the supernatant from the 50 ml tube.
14. Resuspend pellet in 200 µl RPMI-10 (RT) and place on ice.
15. Transfer **100 µl** to a pre-labelled eppendorf vial and wash with 1 ml cold PBS.
16. Spin eppendorf vial at **4°C** 1000g (~3300 rpm) for 5' in an eppendorf centrifuge
17. Remove supernatant from the eppendorf vial and store the pellet at -80°C
18. Take part of the remaining suspension from step 14 for cell counting.
19. Count the cells and bring to a concentration of $2x10^7$ cells/ml by addition of ice-cold RPMI-10.
20. Swirl the tube gently and add drop-wise ice cold freezing medium until the volume is double the volume of the cell suspension ($1x10^7$ cells/ml). Keep as cold as possible on ice.
21. Aliquot in 0.5 – 1 ml aliquots in pre-labelled cryovials (5-10 $x10^6$ cells/vial).
22. Use in-house standard procedure (e.g. use. "Mr Frosty") for freezing cells or place vials in a cell freezer and run program:
    - Quick cool to 0°C
    - Cool -2°C/min to -80°C
23. Store samples preferably in liquid nitrogen. If not available, storage at –80°C is also possible.

2.6 Waste disposal

Adhere to the regulations of your institution.



3 TRANSPORT OF SAMPLES

For shipment to PamGene the samples need to be clearly labelled and packed in a plastic bag or box, including a description of the content in a sufficient amount of dry ice. A digital version of the list of samples is also sent to dpijnenburg@pamgene.com

Shipping address: D.A. Pijnenburg BSc
Manager Research Partnering Group
PamGene International B.V.
Wolvenhoek 10
5211 HH 's-Hertogenbosch
The Netherlands

Contact information: ☎ +31 (0)73 615 89 35
☎ +31 (0)73 615 80 80 (general number)
🖷 +31 (0)73 615 80 81
✉ dpijnenburg@pamgene.com

Please provide tracking number if available.

4 SUPPORT

For questions contact our support group.

Please contact support on:
☎ +31 (0)73 615 89 00
✉ support@pamgene.com

5 RIGHTS AND RESTRICTIONS

5.1 Disclaimer

FOR RESEARCH PURPOSE ONLY

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Notes:

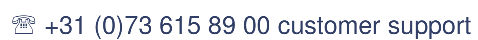
Customer Support

PamGene International B.V.

Wolvenhoek 10

5211 HH 's-Hertogenbosch

The Netherlands



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