Protocol 1161

For shipment preparation of Cell Lines or Purified Cells

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Version 1

Contents

1 INTRODUCTION

1.1 Intended use

The aim of this protocol is to prepare cell pellets from cell lines or purified cells for shipment to PamGene.

2 PROTOCOL

2.1 Materials & reagents

Material/Equipment	Supplier	Catalog number	Storage
PBS: Phosphate Buffered Saline (ice-cold)			

Cells for lysis

Cell culture

- Loosely adherent (recommended 80-90% confluency)
- Suspension cells

Purified cells, e.g.:

- Peripheral Blood Mononuclear Cells (PBMC's)
- Monocytes (*For cells purified from blood, be sure no (or as few as possible) erythrocytes are present, for the large amount of protein in red blood cells disturbs the kinase assay).*

2.2 Equipment

Centrifuge (pre-cooled to 4°C).

2.3 Safety & Precautions

Standard laboratory safety regulations apply.

2.4 Cleaning Tools and Equipment

No special cleaning required.

2.5 Procedure

1. Handle all samples in the same way. Keep all samples as cold as possible by using ice-cold solutions, keeping the samples on ice and pre-cooling all tubes. Typically, PamGene requires 1-2x10^6 cells in a cell pellet for testing conditions. In case of extra multiple replicas and/or inhibitor spike-in experiments, more cells may be required. It is advised to contact PamGene before preparing samples about exact amounts needed for specific studies.

2. Read section 3 Notes AND FAQ before proceeding.

3. Pre-cool centrifuge to 4ºC. Pre-cool all solutions and tubes on ice and work fast (do not process more than 4 samples in parallel).
4. Prepare for every sample tubes with adequate labelling and store on ice.
5. For **suspension cells**, proceed with step 8,
6. For **loosely attached adherent cell**s, proceed with step 12,
7. For **adherent cells**, trypsin treatment to detach cells must be avoided because this influences kinase activity. Instead perform direct lysis by scraping cells in lysis buffer. (see protocol 1160 available from PamGene website)

8. For **suspension cells**, transfer cell suspension(s) to centrifuge tube(s). Centrifuge tubes for 8 min 500 x g at 4°C (In case of unequal volumes, tare tubes with culture medium).
9. Pour off medium carefully in one movement into a beaker. When pellet detaches, stop and centrifuge again. Place tube on ice.
10. Carefully resuspend pellet in 1 ml ice-cold PBS and transfer this to an ice-cold 1.5ml Eppendorf vial and centrifuge for 5 min 1000xg at 4°C.
11. Carefully remove as much supernatant as possible by pipetting without disturbing the pellet and place on ice.

12. For **loosely adhering cells**, pour off the culture medium. Place flask/ plate on ice. Pipette ice-cold PBS in flask/ plate, pipette gently over the cells to detach them. When cells are detached, transfer them to a cold centrifuge tube/ 1.5ml vial.
13. Centrifuge for 8 min 500 x g at 4°C (In case of unequal volumes, tare tubes with PBS).
14. Carefully remove as much supernatant as possible by pipetting without disturbing the pellet, and place tube/ vial on ice.
15. Store samples at –80°C or ship immediately. (preferably snap-freeze samples on dry ice or liquid nitrogen before storage; Use the same procedure consistently for all samples).

2.6 Waste disposal

Use the accepted internal procedures for disposal of tissue residues and for laboratory waste.

3 NOTES AND FAQ

3.1 Notes

1. Biological replicates should be cultured under the same experimental conditions. Samples that will be compared should be cultured and processed under identical conditions.
2. When cells are small, the number of cells per 100 µl lysis buffer can be increased or the volume of lysis buffer can be reduced.
3. In general, the numbers of cells needed per array for the STK assay is about 10,000 cells, and for PTK assay 100,000 – 200,000.
4. When cells have been cultured in the presence of a kinase inhibitor, they should be washed carefully to remove any external inhibitor molecules. Inhibitor taken up by the cells might end up in the lysate and interfere with inhibitor studies. This depends on the cell line and the inhibitor.

3.2 Frequently asked questions on materials & reagents

- *"How can I calculate the correct rotor speed (RPM) to use for pelleting my cells by centrifugation?"*
  g Force or Relative Centrifugal Force (RCF) is the amount of acceleration to be applied to the sample. It depends on the revolutions per minute (RPM) and radius of the rotor. It is relative to the force of Earth's gravity. A good and precise protocol for centrifugation instructs you to use the g force rather than RPMs because the rotor size might differ, and g force will be different while the revolutions per minute stay the same. Centrifuges may have an automatic converter. To convert the g force to rpm for a particular centrifuge you can check whether the supplier of your centrifuge has a conversion tool on its website. Alternatively, you can measure the maximum radius of your rotor and enter the information into the formula:

  g Force (RCF) = (rpm)2 × 1.118 × 10-5 × r
  with
  g = Relative Centrifuge Force
  r = rotational radius (cm)
  N = Revolutions Per Minute (RPM)

4 TRANSPORT OF SAMPLES

For studies conducted by PamGene, please contact PamGene before preparing samples about exact amounts needed for specific studies.
In case of shipment to PamGene, the samples need to be clearly labelled (what is in the tube and date should at least be noted) and packed in a sufficient amount of dry ice accompanied by a list of samples, with preferable an available tracking number.

Shipping address:

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5 SUPPORT

For questions contact our support team.

Contact support:

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6 RIGHTS AND RESTRICTIONS

6.1 Disclaimer

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