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hPBMCs thawing and plating (no specific down-stream assay)

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Protocol status: Working
We use this protocol and it's working

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Abstract

General protocol for thawing and plating human PBMCs from cyrovials.

Materials

- Water bath at 37 degrees (use on in main lab)
- Dry ice
- 1 × 50mL falcon tube per cryovial, with 25mL of thawing media at 37 degrees
- MACs buffer at 37 degrees
- Thawing media at 37 degrees
- Plating media at 37 degrees
- Tissue culture plasticware: 24-well plates, p1000 pipette tips, p20 pipette tips, stripettes, aspirating stripettes, trypan blue, countess slides, eppendorfs

Protocol

1 Retrieve cryovials for plating from Mr W and place on dry ice.

2 Make sure you have 1 falcon with thawing media per cryovial ready in the hood, pre-warmed to 37 degrees.

3 Place cells in floaty and place in water bath and allow to thaw for approx. 90 seconds.

4 Check to see if thawed, a small amount of ice remaining is fine.

5 Pour contents of cryovial into falcon containing thawing media.

6 Centrifuge falcons at room temp for 10 minutes at 300 x g.

7 Aspirate supernatant and resuspend pellet in 10mL MACs buffer – make sure resuspend pellet first in 1mL of warmed MACs with a p1000 pipette to ensure pellet is broken up, then add 9mL MACs with stripette.

8 Take 10uL of cell suspension and place in Eppendorf.

9 Centrifuge falcons 300 x g 10 mins at room temp.

10 Whilst falcons are centrifuging proceed with cell count: mix 10uL of trypan blue with 10uL of cell suspension, place 10uL in both chambers of a countess slide and count using “hPBMC cryo” programme on countess.

11 Note cell count per mL (countess accounts for 1:1 trypan blue dilution, so this number is accurate already) and live cell % (aiming for 80+ % viability).

12 Calculate volume of plating media needed for each pellet to obtain 1 × 10^6^ cells per mL.

13 Example: countess count = 5 × 10^5^ per mL. 5 × 10^5^ x 10 (as cells were in 10mL) = 5 × 10^6^ cells total. Therefore resuspend cells in 5mL for 1 × 10^6^ per mL.

14 For each patient sample, plate 500uL of cell suspension per well of a 24-well plate, and plate 1 well per treatment required for experiment.
NOTE: This can be adjusted based on downstream assay being used. E.g. flow cytometry = 1 million/mL and plate 500uL in 24 well plate (results in 500,000 per well), whilst RNA needs more cells so plate 2mL in 6 well plate (results in 2 million per well).

15 For each patient, label wells with patient ID and treatments.

16 Place in incubator for MINIMUM of 2 hours to rest and proceed with downstream assay.