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Procedure for thawing Poietics™ cells

DNase I functions to prevent clumping of culture cells, and mononuclear cells, and should be added to medium.

1. Warm medium containing 10% FBS or 1% BSA. For mononuclear cells, add DNase I at 20 U/ml (*see below).
2. Quickly thaw the vial of frozen cells in a 37℃ water bath. Wipe the outside of the vial with 70% ethanol.
3. Aseptically transfer a maximum of 2 ml of cell suspension to a 50 ml conical tube. For one million cells or less, use a 15 ml conical tube.
4. Rinse the vial with 1 ml of medium. Add the rinse dropwise to the cells while gently swirling the tube (≈ 1 minute).
5. Slowly add enough medium dropwise to the cells until the total volume is 5 ml, while gently swirling after each addition of several drops of medium (≈ 3 minutes).
6. Slowly bring the volume up to fill the tube by adding 1 ml to 2 ml volumes of medium dropwise, while gently swirling after each addition of medium (≈ 5 to 10 minutes).
7. Centrifuge the cell suspension at 200 X g at room temperature for 15 minutes.
8. Carefully remove most of the wash by pipette (and save in a second tube), leaving a few milliliters behind so the cell pellet is not disturbed. Gently resuspend the cell pellet in the remaining medium. If you are using a 50 ml tube, transfer the cells to a 15 ml conical tube and rinse the 50 ml tube with 5 ml of medium. Slowly add the 5 ml wash medium to the cell suspension with gentle swirling.
9. Slowly bring the volume up to fill the tube by adding 1 ml to 2 ml volumes of medium while gently swirling after each addition of medium.
10. Centrifuge the cell suspension at 200 X g at room temperature for 15 minutes.
11. Carefully remove by pipet all but 2 ml of the wash. Gently resuspend the cell pellet in the remaining 2 ml of medium and count. If cell count is lower than expected, centrifuge wash saved in step 8 at a higher speed, count and combine if necessary.
12. Rest the cells for 1 hour at 37℃ and 5% CO_2. Count the cells a second time. The cells are ready to be put in culture.

*For the addition of DNase, prepare 20 ml of medium containing 10% FBS and 20 U/ml of DNase I (Sigma D 4513). Proceed as above, using the DNase-containing medium to dilute the cells. Centrifuge the cells and continue with step #8.