stemexpress®
advancing science
1743 Creekside Drive, Suite 200
Folsom, CA 95630
530.626.7000
email: info@stemexpress.com
www.stemexpress.com

Human Peripheral Blood Leukopak Thawing Protocol

StemExpress Peripheral Blood Leukopaks are carefully frozen in a medium containing the cryoprotective agent DMSO (10%) using a controlled rate freezer to ensure maximum viability. Outlined below is our in-house procedure for thawing cells to maintain a high viability and recovery. When using this procedure or any thawing procedure, it is important to work quickly as cryopreserved cells are extremely fragile and require the utmost care. Although DMSO protects the cells while frozen, it is toxic to the cells at room temperature for even a short period of time.

Work fast and be gentle to your cells!

Materials Required

- Frozen cryobag containing cells
- Thawing Medium: HBSS or PBS without calcium or magnesium, 10% heat inactivated FBS warmed to 37°C (Replace 10% FBS with 10% HSA for animal component free medium)
- Buffer: PBS without calcium or magnesium, heat inactivated 0.5% BSA or 0.5% HSA, and 2 mM EDTA
- Sterile 50 mL centrifuge tubes
- 70% ethanol
- Sterile 500 mL container

Equipment

- Water bath warmed to 37°C
- Centrifuge
- 25 mL pipette
- Pipettor

Protocol

1. Warm Thawing Medium in 37°C water bath.
2. Place the cryobag in 37°C water bath. The time taken to thaw the cells will vary depending on the volume within each cryobag. Be careful not to submerge the ports on the cryobag.

   Note: Perform this step immediately after removing the cryobag from the dry ice in the shipment or after removing the cryobag from your liquid nitrogen storage.
3. Remove the cryobag from the 37°C water bath when small ice crystals remain.

   Note: Prolonged exposure to heat will damage the cells and increase cell death.
4. Remove the cryobag from the water bath to a biosafety hood. Clean the outside of the cryobag with 70% ethanol.
5. Cut the cryobag with sterile scissors and add an equal volume of Thawing Medium dropwise to the cryobag.

   Note: Adding Thawing Medium dropwise is gentler on stressed cells and can help increase cell viability. Avoid any type of agitation to the cells.
6. Gently transfer cells to a 500 mL container. Leave enough extra volume for the addition of the Thawing Medium.
7. Add 3-4 times the volume of Thawing Medium dropwise to the 500 mL container.
8. (Optional) Rinse the cryobag with 25 mL warm Thawing Medium and transfer to the container.

   Note: Rinsing the cryobag will help recover remaining cells.
9. Use a small aliquot to determine viability and cell count.
10. Transfer cells to 50 mL conical tubes using a 25 mL pipette.
11. Centrifuge the 50 mL conical tubes at 300 x rcf for 10 min (accel 5, brake 0).
12. Remove the supernatant and gently resuspend the cells using a total of 40 mL Buffer and combine the cells.
13. Gently swirl the cell suspension to make sure cells are equally distributed in the solution. Failure to mix well will result in inaccurate cell counts and viability testing.

    Note: Do not use a vortex mixer to resuspend cells. This will damage the cells and decrease viability.
14. Use a small aliquot to determine viability by PI or trypan blue staining and for cell counting.

    Note: It is important to check the viability of your cells, as a low viability can interfere with downstream applications.
15. Depending on the sensitivity of the cell type, allow them to recover in an appropriate medium for 24hrs.

If you have any questions please contact us at 888-415-4215 or 530-303-3828.

Important:
Make sure the viability and cell count is performed throughout the thawing protocol.

THE PURITY, VIABILITY & QUALITY YOU NEED™