Cryopreserved Leukopak Thawing

PROTOCOL

Materials

- Conical tubes, 50 mL
- Serological pipettes, 5–20 mL
- Media bottle, 500 mL or 1 L
- Connecting set (e.g., OnGuard; optional)
- Single channel pipette with sterile tips
- Cell counting slide
- Sterile scissors

Reagents

- 70% Ethanol
- Fetal bovine serum (FBS)
- Salt solution (e.g., Hanks Balanced Salt Solution, HBSS)
- DNase (optional)

Equipment

- Biological safety cabinet (BSC; BSL-2)
- Temperature-controlled swinging bucket centrifuge
- 37°C water bath

Procedure

Considerations

All procedures are performed with Universal Safety Precautions using appropriate PPE, aseptic technique, and under sterile conditions in a BSC.

All samples are considered potentially infectious (BSL-2 or higher).

Further processing or modification of the steps in this protocol may be necessary, depending on the application.

Thawing and washing are expected to negatively impact total cell counts and viability.

Preparation

1. Turn on the biosafety cabinet and ensure a sterile environment, spraying all materials, equipment, and reagents entering the cabinet with 70% ethanol.
2. Prepare fresh thawing media by adding supplementing the salt solution with 10% FBS. DNase at a final concentration of 0.1 mg/mL is optional (Note: do not add DNase if the cells will be used for DNA or RNA extraction).
3. Prepare a 37°C water bath.

Thawing

4. Remove the leukopak from cold storage and its freezing cassette.
5. Transfer the leukopak to the water bath, careful not to submerge the ports.
6. Transfer the leukopak to the BSC when the product is mainly thawed (i.e., 10% is still visibly frozen).
7. Transfer the contents of the leukopak to a sterile bottle capable of containing at least five times the leukopak original volume.
   a. The transfer is best accomplished utilizing the spike port and a transfer set, if available.
   b. If a transfer set is unavailable, use sterile scissors to cut the tubing and pour the contents into the sterile container.

Wash 1

8. Add thawing media to the container 1:1 with leukopak contents by slowly dispensing the thawing media and swirling the suspension simultaneously.
9. OPTIONAL: rinse the leukopak bag with ~50% of its original volume of thawing media to collect any remaining cells.
   a. If you use a transfer set, be sure the clamp is closed.
   b. Without a transfer set, fold the bag to ensure the leukopak does not leak while adding the thawing media.
10. OPTIONAL: cut across the top section of the leukopak with sterile scissors and add thawing media to the bag. Gently mix the bag's contents by folding the bag to prevent leakage and rocking the bag side to side.
    a. If using a transfer set, fold the top half of the bag where the cut was made to avoid leaking.
    b. In the absence of a transfer set, fold both ends of the bag.
11. Slowly add two volumes of thawing media to the suspension. Note the total volume of suspension.
12. OPTIONAL: mix the suspension gently and set aside a small sample for counting.
    a. Count the cells using your preferred method during the centrifugation step below.
13. Transfer the suspension to 50mL conical tubes, splitting as evenly as possible.
14. Centrifuge at 300 x g at room temperature for 10 minutes with the low brake on.
15. Transfer the tubes to a BSC and discard the supernatants, careful not to disturb the cell pellets.
16. Resuspend the pellet in the residual supernatant.
    a. NOTE: If the cells are beginning to clump, add 0.1 mg DNase per ml of cell suspension and incubate for 15 minutes at room temperature (15-30°C).

Wash 2

17. Top up each conical tube to 50 mL with thawing media.
18. Centrifuge at 300 x g at room temperature for 10 minutes with the low brake on.
19. Transfer the tubes to a BSC and discard the supernatants, careful not to disturb the cell pellets.