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Standard Process for Thawing Viable Cell Products

Reagents:

- **Culture Media** (Specific for Downstream Application)
- **Benzonase** (if needed)
- **Discovery Life Sciences Viable Cells** (PBMCs, BMMCs, DTCs)
    - All Discovery Life Science Viable Cell Products are shipped on dry ice and should be used immediately or stored in liquid nitrogen. For more information, please refer to “Recommended Handling and Storage for Biological Specimens”

Equipment:

- **37°C Water Bath**
- **Cell Counter**
- **Centrifuge**
- **Micropipettes**
- **Pipettors**

Procedure:

1. Warm culture media to room temperature prior to thawing cells.

2. Dispense 9mL of media into a 15mL conical tube (Prepare as many of the 15mL conical tubes as there are vials to thaw).

    For viable cell products with cell counts less than 3x10^6 cells, dispense 1-4mL media to ensure that the cell counts are within the linear range of the cell counter being used.

3. To thaw cells, gently swirl the vial of cells in a 37°C water bath until only a small frozen pellet remains.
4. Alcohol the outside of the vial and quickly transfer into a biosafety hood.

5. Using a P1000 micropipette, slowly and gently transfer the cell suspension from the vial into the 15 mL conical tube containing media.

Standard Process for Thawing Viable Cell Products - Continued

6. Gently rinse the vial with 1 mL of the culture media from the 15 mL conical tube.

7. Count cells using preferred method.

   Note: Dissociated tumor cells (DTCs) may have a significant amount of debris. Please refer to the FAQs for any automated cell counter being used for any potential issues regarding cell suspensions with high debris content. If possible, an automated cell counter that utilizes multiple DNA dyes to identify live and dead nucleated cells from debris, such as the Nexcelom Cellometer Vision CBA Image Cytometer or Millipore Guava easyCyte Flow Cytometer, is highly recommended. It is not recommended to use trypan blue exclusion methods for counting DTCs.

   *** If excessive cell clumping is observed, follow the protocol below in lieu of steps 8 and 9.*

8. Centrifuge cell suspension at 300xg for 10 minutes at room temperature.

9. Resuspend cells in cell culture media and proceed with downstream applications

**** Optional Protocol for Samples with Cell Clumps:**
If excessive cell clumping is observed, the following steps can be performed ***after step 7***

1. Resuspend cells in 10 mL cell culture media containing 10-25U/mL benzonase.

2. Centrifuge cell suspension at 300xg for 10 minutes at room temperature.

3. Resuspend cells in fresh 10 mL cell culture media containing 10-25 U/mL benzonase.

4. Centrifuge cell suspension at 300xg for 10 minutes at room temperature.

5. Resuspend cells in cell culture media and proceed with downstream applications.