All vials of frozen cells should be stored in the vapor phase liquid nitrogen (≤-150°C). When needed for use, transfer up to 4 vials from the vapor phase liquid nitrogen to the lab on dry ice to keep them cold. Only thaw 4 vials at one time.

1. Transfer frozen vials to the lab on dry ice to keep them cold. Only thaw 4 vials at one time.
2. Warm media for cell dilution to 37 ± 3°C. For each vial to be thawed, place 10 mL of media in a 50 or 15 mL centrifuge tube.
3. Thaw the frozen vials in water in a 37 ± 3°C water bath with gentle shaking. Just before the last ice crystal has melted, remove the vial from the water. Wipe the vial with a sterile alcohol pad, focusing on the cap area.
4. Add 1 mL of warm culture media from the 50 or 15 mL tube prepared above to the vial using a 1000 µL pipette tip and drop-wise action. Add the culture media over 30 sec to allow the cells to adjust to the change of temperature.
5. Slowly transfer the diluted cells back to the 50 or 15 mL tube. Do not mix or pipette the cells vigorously since cells that have been frozen are initially more sensitive to mechanical stress than fresh cells.
6. Rinse the original vial with 1 mL of the cell containing media from the 50 or 15 mL tube to recover cells that may have adhered to the sides; add the rinse media back to the 50 or 15 mL tube.
7. Pellet the cells by centrifugation at 350xg for 10 minutes with rapid acceleration and brake on. If no pellet is observed, centrifuge at 450xg for an additional 15 minutes.
8. OPTIONAL STEP, PROCEED DIRECTLY TO STEP 9 IF PLANNING TO OMIT DNase TREATMENT: To avoid any issues with potential cell clumps, resuspend the cell pellet in a small volume of warm media (1-2mL) and mix by gently tapping. Add 100µL of 1mg/mL DNase I. Mix gently but thoroughly and incubate at room temperature for 15 minutes. Then bring the total volume to the desired counting volume using warm media, mixing the cell solution carefully. Note that if using these optional instructions, DNase does not need to be washed out of the solution carefully. Pellet the cells by centrifugation as in step 7.
9. Re-suspend the cell pellet by gently tapping and add 10 mL of warm culture media for a second wash. Mix cell solution carefully. Pellet the cells by centrifugation as in step 7.
10. Discard supernatant.
11. Count cells using laboratory specific procedures and proceed with laboratory protocol assays.

Thawed cells may benefit from resting overnight at 37 ± 3°C before use in assays. Adjust viable cell concentration to 2 x 10⁶ cell/mL. Put 1-10 mL of cell suspension in a 50 mL conical tube, loosen the tube cap to allow for gaseous exchange, and place the tubes upright in an incubator with the appropriate CO₂ level for the media used. After overnight incubation, count and use cells as needed.

Use Universal Precautions for handling cellular products as for other human specimens. Do not pipette by mouth. Avoid direct inhalation of the suspension and handle in areas with adequate ventilation. Do not smoke, eat or drink in areas where specimens are being handled. Dispose of this product as appropriate for biohazardous material.

For Research Use Only. Not for use in diagnostic or therapeutic procedures.

INSTRUCTIONS FOR THAWING AND CULTURE