

Xenografting Human Primary Samples

PURPOSE:

This document establishes a protocol for thawing and subcutaneously injecting tumor dissociated human primary cells obtained from Conversant Bio into mice.

THAWING PROCEDURE:

- 1) Quickly thaw sample in a 37 - 40C water bath until a ~2 mm crystals remains. Move sample through the water to speed thawing.
 - a. NOTE: samples are cryopreserved in media containing 10% DMSO.
- 2) Spray the sample with 70% ethanol and dry using a ChemWipe.
- 3) Transfer cells from the cryovial to a 50 mL conical containing 500 μ l of PBS or cell culture media. Rinse the inside of the cryovial with PBS or cell culture media
 - a. Cell count and viability can be measured at this point by mixing 10 μ l of cells with 10 μ l of Trypan blue (or alternative dilution) and examining the cells on a hemocytometer.
 - i. Recommended if the expected cell number is near 1 million cells or less per mL.
 - b. Quickly move to the next step since prolonged exposure to DMSO is toxic to cells.
- 4) Very slowly add 10-20 mL of PBS or cell culture media that was previously equilibrated to 37C to the conical with constant stirring.
- 5) Fill the conical with PBS or cell culture media to further dilute the DMSO.
- 6) Centrifuge the conical at 300xg for 5-10 minutes and remove the supernatant.

SUBCUTANEOUS INJECTION PROCEDURE:

- 7) Tap the conical to resuspend the cells using the residual PBS or cell culture media above the cell pellet.
 - a. Residual PBS or cell culture media should be 100-150 μ l.
 - b. Do not vortex the cells.
 - c. Some cell clumping is acceptable
- 8) Place the conical with cells on ice.
- 9) Using a previously chilled 1 mL syringe, add 150-200 μ l of chilled extracellular matrix (recommend: BD Matrigel™ Basement Membrane Matrix High Concentration, cat# 354262).

- a. If using BD Matrigel™:
 - i. Thaw a frozen vial of BD Matrigel™ overnight at 4C.
 - ii. Thoroughly mix contents by inverting the vial several times.
 - iii. Keep cold at all times to prevent the gel from solidifying.
- 10) Tap the conical several times to homogenize the cells with the extracellular matrix.
 - a. Do not vortex.
 - b. Keep cold.
- 11) Take up the extracellular matrix / cell mixture into a previously chilled 1 mL syringe.
- 12) Inject the mouse subcutaneously with the bevel of the needle pointing up.
- 13) Create a subcutaneous space by moving the tip of the needle side-ways several times.
- 14) Slowly eject the contents of the syringe into the subcutaneous space and wait a few seconds for the matrix to start solidifying.
- 15) Carefully pull-out the needle to limit leakage.