Tumor Dissociation Protocol – Modified Miltenyi Biotec

Materials:

1. RPMI 1640 (without FBS)
2. RPMI 1640 (with 10% FBS)
3. 6-well plates
4. 96-well plates
5. Tumor Dissociation Kit, Human
    a. Enzyme H – 200 µl/vial
    b. Enzyme R – 100 µl/vial
    c. Enzyme A – 25 µl/vial
6. gentleMACS C Tubes
7. gentleMACS Octo Dissociator with Heaters
8. Hemocytometer
9. Trypan Blue
10. 1000 µl pipette
11. Pipette tips
12. Carbon Steel Surgical Blades
13. 100 micron nylon strainers
14. Hood vacuum
15. 50mL Conical Tubes
16. Centrifuge
17. Lysis Buffer
18. Eppendorf Tubes
19. Bambanker solution
20. Mr. Frosty cell freezing flask
21. LN_2

Refer to the Tumor Dissociation Kit, human (Miltenyi Biotec, 130-095-929) protocol for additional information.

Prior to dissociation, reconstitute lyophilized powder of each enzyme in the Tumor Dissociation Kit and prepare aliquots. Store at -20˚C.

Enzyme H – 3mL RPMI 1640 (w/o FBS); 200µL aliquots

Enzyme R – 2.7mL RPMI 1640 (w/o FBS); 200µL aliquots

Enzyme A – 1mL Buffer A (do not vortex); 25µL aliquots

Enzymes and cells should be kept on ice throughout the protocol and sterile technique should be used.

Procedure:

1. Weigh the specimen on a petri dish and record. One portion of RPMI/enzyme mix and one gentleMACS C tube is required for each 1g of tumor.
2. Thaw the required number of pre-aliquoted, frozen Enzyme H, Enzyme R, and Enzyme A.
3. Take 5mL of RPMI (without FBS) and mix with the three different enzymes from the tumor dissociation kit in one well in the 6-well plate.
4. Use a new surgical blade to dice the tissue into 2-4mm pieces in the RPMI/enzyme solution in the 6-well plate.
5. Using a 1000mL pipette, transfer the entire solution containing tissue and media into the gentleMACS C tube.
6. Place the gentleMACS C tube upside-down in the gentleMACS Octo Dissociator with Heaters unit ensuring the sample material is in the area of the rotor and set it to the desired program based on tumor type. Allow to run for 30 minutes.
    a. For soft tumors (ovarian, colon, renal) – Program 37C_h_TDK_1
    b. For medium tumors (lung, prostate) – Program 37C_h_TDK_2

c. For hard tumors (breast, pancreatic, head and neck) – Program 37C_h_TDK_3

Refer to the Miltenyi gentleMACS Octo Dissociator with Heaters manual for additional information.

7. Pre-wet a 100-micron nylon strainer set up in a 50mL conical tube with RPMI containing FBS. After the 30-minute run time, pour the solution from the gentleMACS C tube slowly through the strainer into the 50mL conical tube. If there is tissue stuck in the strainer, use the flat surface of a blade handle to mash it through the strainer. Wash with more RPMI containing FBS until the 50mL conical tube is filled. *Note: if solid pieces remain or clumping occurs, restrain by passing solution through strainer a second time without mashing*
8. Spin at 500xg for 5 minutes (this may change dependent on cell type).
9. Once spun down, aspirate the RPMI and leave the cell pellet at the bottom. If red blood cells are present (i.e. if the pellet is tinted red), use a lysis buffer to remove them as per the manufacturers instructions.
10. Resuspend the pellet in 1mL of RPMI containing 10% FBS in the 50mL conical tube (the amount of RPMI used depends on the size of the pellet – for a large, visible pellet ≥ 3-4mm, resuspend in a higher volume)
11. Pipette 10 µl of the solution into 3 different wells in a 96-well plate or appropriately labeled Eppendorf tubes.
12. Dilute the first well with Trypan Blue. The dilution ratio will depend on the size of the pellet – a large pellet will require a higher dilution.
13. Place the solution on the hemocytometer. Count live cells and calculate the total amount of cells in the solution.
14. Once the cells have been counted, fill up the 50mL conical tube containing the cells with RPMI containing 10% FBS. Centrifuge for 5 minutes at 500x g.
15. Aspirate the RPMI, leaving behind the pellet.
16. Resuspend the pellet in bambanker solution to a concentration of ≥ $1x10^6$ cells/mL. Transfer 1mL aliquots of cells in bambanker to labelled cryovials and place in a Mr. Frosty cell freezing flask. Store at -80˚C.
17. Transfer cryovials to LN_2 after 3 days.