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Tumor Dissociation to Single Cell Suspension

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We use this protocol and it's working

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Abstract

This protocol has been used to generate a single cell suspension from tumor tissue for use in single cell analysis. It has been optimized to eliminate dead cells that may be present after cancer therapy and which can compromise single-cell RNAseq and other assays that require a high proportion of live cells.

Troubleshooting

Materials

1

- Tumor Dissociation Kit, human (Miltenyi Biotec, 130-095-929)
- RPMI 1640 or DMEM media
- MACS SmartStrainers (70 µm, 30 µm) (Miltenyi Biotec, 130-098-462, 130-098-458)
- gentleMACS Octo Dissociator with Heaters (Miltenyi Biotec, 130-096-427)
- gentleMACS C Tubes (Miltenyi Biotec, 130-093-237, 130-096-334)
- Red Blood Cell Lysis Solution (10×) (Miltenyi Biotec, 130-094-183)
- Dead Cell Removal Microbubble Kit (Akadeum Life Sciences, 11510-211)

Tissue Collection

2

1. Euthanize mice and excise tumors with sterile instruments
2. Remove all excess skin, fat, connective tissue, and necrosis if necessary
3. Weigh cleaned tumors and record mass
4. Place tumor piece in sterile 1.5 mL microfuge tubes with 200 µL plain DMEM or RPMI 1640 media and place on ice
5. Repeat steps 1-4 for all desired tumors

Tissue Dissociation

3

1. Follow protocol from Tumor Dissociation Kit, human (Miltenyi Biotec, 130-095-929) as of 5/6/2024 to 2.2.1, step 1

2. Check dissociation progress of samples halfway through protocol
if milky with minimal visible tumor pieces left, stop program to prevent over-dissociating
if pink/clear with many visible tumor pieces left, continue program till end

3. If continued program to end, check dissociation progress again using above metrics
if still pink/clear with many visible tumor pieces left, repeat program and check halfway through again

4. Once solution is milky with minimal visible tumor pieces left, stop program remove C tube
Place 70 um smart strainer on 50 mL tube and pre-wet filter with 2 mL of plain DMEM or RPMI media

5. Using serological pipette, transfer solution in C tube to 50 mL tube with strainer

6. Wash down C tube with 20 mL of plain DMEM or RPMI 1640 media and transfer to same 50 mL tube with strainer

7. Centrifuge 50 mL tube at 300 x g for 7 minutes

Proceed to clean up while samples are in centrifuge

Clean-up

4 1. Make red blood cell lysis solution by combining materials below and leaving out at room temperature

Per sample:

4.5 mL double distilled water (room temperature)

0.5 mL red blood cell lysis solution (10x)

2. Aspirate supernatant without disturbing pellet

3. Add 1 mL of prepared red blood cell lysis solution and resuspend pellet using the least amount of force as possible

4. Add additional 2-4 mL of prepared red blood cell lysis solution depending on pellet size and presence of red blood cells

5. Vortex for 5 seconds and incubate samples for 2 minutes at room temperature

7. Centrifuge samples at 300 x g for 7 minutes

if red blood cells still visible repeat steps 1-7

8. Aspirate supernatant without disturbing pellet

9. Resuspend pellet in plain DMEM or RPMI 1640 media (80-500 L depending on pellet size, want to use smallest volume of media as possible while still fully resuspending pellet with minimal force)

10. Count using countess or hemocytometer (1:1 cell solution:trypan if small pellet, 1:1 cell solution:media then 1:1 diluted cell solution: trypan if large pellet)

11. While counting check for dead cells and cellular debris

If acceptable viability (>80%), proceed with sample for subsequent experiments

If unacceptable viability (<80%) and large presence of cellular debris, proceed with additional clean up

Additional Clean-up

5 **If sample has large presence of debris**

1. Dilute cell solution to total of 5 mL with plain DMEM or RPMI 1640 media

2. Place 30 um smart strainer on 15 mL tube and pre-wet filter with 2 mL of plain DMEM or RPMI 1640 media

3. Using serological pipette, transfer solution to 15 mL tube with strainer

4. Centrifuge at 300 x g for 7 minutes

5. Aspirate supernatant without disturbing pellet

6. Resuspend pellet in plain DMEM or RPMI 1640 media (80-500 µL depending on pellet size, want to use smallest volume of media as possible while still fully resuspending pellet)

7. Count using countess or hemocytometer (1:1 cell solution:trypan if small pellet, 1:1 cell solution:media then 1:1 diluted cell solution: trypan if large pellet)

8. While counting check for dead cells and cellular debris

If acceptable viability (>80%), proceed with sample for subsequent experiments

If unacceptable viability (<80%) and large presence of cellular debris, proceed next clean up steps

5.1 **If sample has small presence of debris but unacceptable number of dead cells**

1. Follow Dead Cell Removal Microbubble Kit (Akadeum Life Sciences, 11510-211) protocol as of 5/6/2024

2. Resuspend cell pellet in 80-200 µL plain DMEM or RPMI 1640 media depending on pellet size, avoiding Microbubbles on side of tube

12. Transfer solution to clean tube

13. Count using countess or hemocytometer (1:1 cell solution:trypan if small pellet, 1:1 cell solution:media then 1:1 diluted cell solution: trypan if large pellet)

14. While counting check for dead cells and cellular debris

If acceptable viability (>80%), proceed with sample for subsequent experiments

If unacceptable viability (<80%) and large presence of cellular debris, proceed repeat steps 6.1 1-14 till acceptable viability is reached