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| Tissue dissociation **Using human tumor dissociation kit from Miltenyi**  **Procedure:**   1. The tissue (~5 mm3) is supplied in 5 ml of Tissue Storage Solution in a 50 ml tube on ice.   Other procedures, including centroifugation, are performed at RT, unless noted otherwise.   1. Put a drop of 200 µL the Enzyme Mix at the corner of a tissue culture plate, which is secured to be held at the 45° angle, and place the tissue in the drop. Cut/chop into as small pieces as possible using scissors and scalpel.   *Remove fat, fibrous, and necrotic areas from the tissue.*  *Transfer (see point 3) the remaining tissue in a tube with Tissue Storage Solution to keep it until you are sure to have the required yield of cells. Mouse lung tissue is stable in this solution for up to 6 hours.*  *Put the leftover in a cryo vial with <1mL of Freezing Medium (kept on ice) and keep in Mr. Frosty™ Freezing Container at -80°C overnight. Then, transfer to a liquid N2 tank for storage for future research. If the tissue is already dissociated, the cells can be snap frozen in N2 or -80*°C *ethanol.*   1. Transfer the 200 µL of the chopped tissue to a 2 mL tube using a 1000-µL Gilson pipet with cut-off tip. Collect the leftovers from the plate two times with a half of the prepared Enzyme Mix (each time), transferring to the 2 mL tube. Tightly close the tube and put it in the most outside extremity (to increase rpm) of the rotator at 37°C.      1. Incubate sample for 15 minutes at 37°C with maximum rotation, pipetting it up and down (3x) for 2 min. every 5 minutes using a 1000-µL Gilson pipet tip (without cut off). After 15 min., remove the tube and vigorously resuspend the sample using a 1000-µL Gilson pipet tip for 2 min until you see no more macroscopic chunks.   *Checkpoints:* Counting with Trypan Blue - use a 10 µL aliquot.   1. Place a strainer in a 50 mL tube and pre-wet the strainer with 1 mL DMEM. Apply the cell suspension to the strainer, collecting the content by gravity. 2. Wash the strainer with 12 mL of DMEM, collecting the content by gravity.   *Before discarding the strainer, make sure that the cells of interest are not retained in the strainer. Place the strainer in a 30 mm dish, fill it in with 1 mL PBS, collect the liquid while pipetting up and down and count a cell aliquot by hemocytometer.*  *Checkpoints:* Counting with Trypan Blue - use a 10 µL aliquot.  Immunofluorescent labeling – use a 200 µL aliquot, to which add 24 µL paraformaldehyde (upto ~4 % paraformaldehyde) for 20 min. After that, centrifuge, remove the fixative and keep the sample in PBS until permeabilization and staining.  *If multiple experimental procedures are pursued in parallel, split the sample in several tubes before proceeding with the centrifugation. Use the appropriate cell number for each and appropriate tubes.*   1. Centrifuge the cell suspension at 300×g for 7 min. Collect the supernatant in a separate tube. 2. Resuspend the cell pellet in the appropriate Buffer, such as PBS/0.04% BSA or for further enrichment, ACK lysis buffer.   *Any leftover can be mixed up with the Freezing Medium (on ice) and frozen.*   1. **ACK lysis**   **Based on the protocol from Lonza** [**https://bioscience.lonza.com/lonza\_bs/US/en/Culture-Media-and-Reagents/p/000000000000181463/ACK-Lysing-Buffer-(1X)**](https://bioscience.lonza.com/lonza_bs/US/en/Culture-Media-and-Reagents/p/000000000000181463/ACK-Lysing-Buffer-(1X))   * 1. For the pellet volume around 1 mm3, use 1 mL of ACK lysis buffer. Perform the treatment in a 15 mL tube. Homogenize the pellet by pipetting 10 times with a 1000-µL cut-off tip. Keep the sample on a bench for up to 2 min.   2. Fill the tube with 10 mL PBS/0.04% BSA. Centrifuge at 300×g for 5 minutes. Check the redness of the pellet. Remove the supernatant using a serological pipet first and then the leftovers with a 200-µL tip, while leaving the cell pellet intact.   *If the pellet is still red, repeat the process one more time.*   * 1. Resuspend the cell pellet in an appropriate solution.   *Checkpoints:* Counting with Trypan Blue.  Immunofluorescent labeling.  ***Proceed straight to* *scRNA-seq or***  ***preliminary cell separation by magnetic beads, in order to enrich for cell of interest*** | 05/15/2019  **Reagents and Checkpoints:**  21.4 ml DMEM at RT (room temperature). Make the aliquot in 1.5 ml tube for the Enzyme Mix to keep at 37°C.  MACS Tissue Storage Solution, 100 ml (Miltenyi #130-100-008)  Miltenyi Tumor Dissociation Kit, human  (Miltenyi #130-095-929)  Enzyme H; Enzyme R; Enzyme A  Scissors, Tweezers, Scalpel  Strainer (40 μm)  **Enzyme Mix**  Prepare in 1.5 mL tube.  50 μL Enzyme H  25 µL Enzyme R  6.25 μL Enzyme A  1119 µL DMEM (corning)  PBS/0.04% BSA  10 mL:  9.99 mL 1x PBS  40 µL BSA 10% (Sigma, # A1595 Sigma, fridge Max lab)  ACK lysing buffer (Lonza # BW10548E)  *Other:*  Freezing Medium:  90% FBS  10% DMSO  37% Formaldehyde Solution (Fisher)  Mini Incubator Labroller Rotator Combo, Labnet International |