DEMONSTRATED PROTOCOL

Tumor Dissociation for Single Cell RNA Sequencing

Overview

This protocol outlines best practices for dissociation of mouse melanoma, color tumor, and breast tumor for use in 10x Genomics Single Cell RNA protocols. Modifications to this protocol (e.g. dissociation time, resuspension buffer, enzyme concentration, centrifugation speed and time) may be required when working with other tumor types.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices and Technical Note Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (Document CG00091) for more information on determining accurate cell counts.

Cells carry potentially hazardous pathogens. Follow material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage, and disposal of biological materials.

Cell Sourcing

Tumor/ Species	Description	Supplier	Part Number
CT26 colon/ mouse	Whole colon tumor in Miltenyi tissue storage solution Tumor size: 300-500 mm^3	Charles River Laboratories	CT26
4T1-e274 breast/ mouse	Whole breast tumor in Miltenyi tissue storage solution Tumor size: 300-500 mm^3	Charles River Laboratories	-
B16F10 melanoma/ mouse	Whole colon tumor in Miltenyi tissue storage solution Tumor size: 300-500 mm^3	Charles River Laboratories	B16F10

Preparation - Buffers

Tumor Dissociation	
Reagents	**Composition**
Enzyme D*	1 vial lyophillized Enzyme D in **3 ml** RPMI 1640/DMEM
Enzyme R*	1 vial lyophillized Enzyme R in **2.7 ml** RPMI 1640/DMEM Thoroughly mix reconstituted Enzyme R immediately before withdrawing the required reaction volume.
Enzyme A*	1 vial lyophillized Enzyme A in **1 ml** RPMI 1640/DMEM
Red Blood Cell Lysis (maintain at 4°C)	
Buffers	**Composition**
Wash Buffer	0.04% BSA in 1X DPBS
Red Blood Cell Lysis Solution	1X Red Blood Cell Lysis Solution in double distilled water DO NOT use deionized water. Discard unused solution at the end of the day.

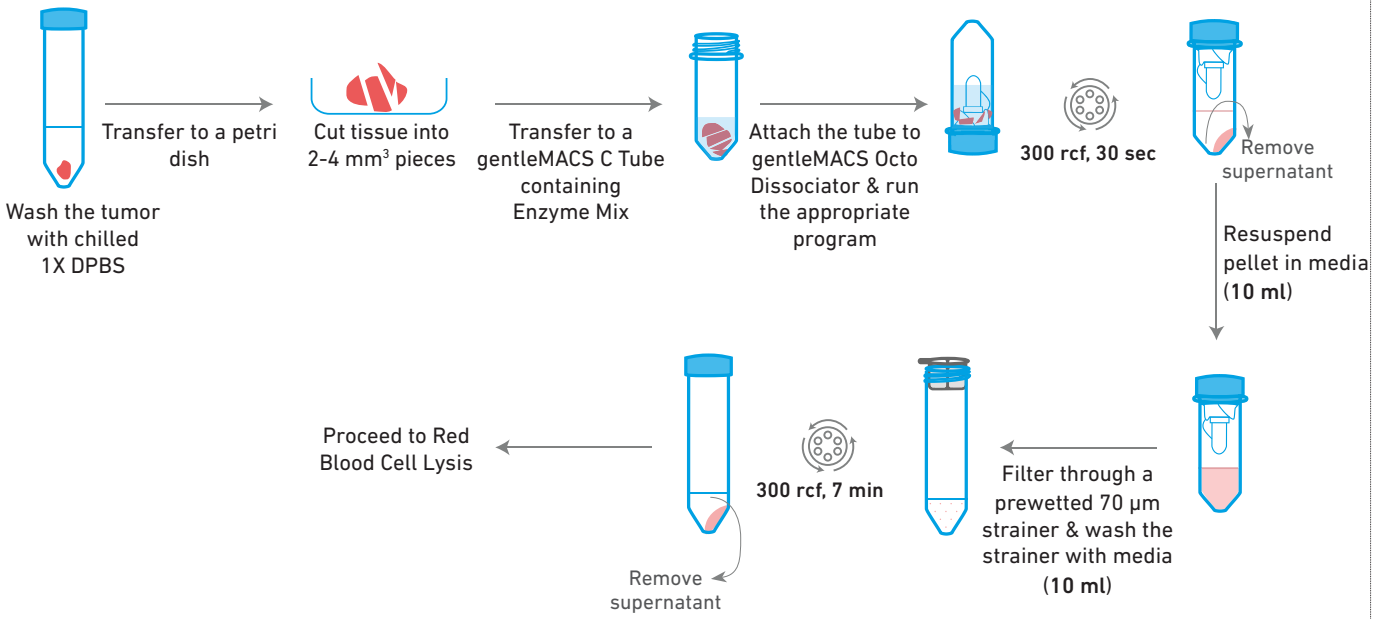
*Part of Tumor Dissociation kit. Aliquot reconstituted enzymes and store at -20°C. Reconstituted enzyme is stable for 6 months.

Specific Reagents & Consumables

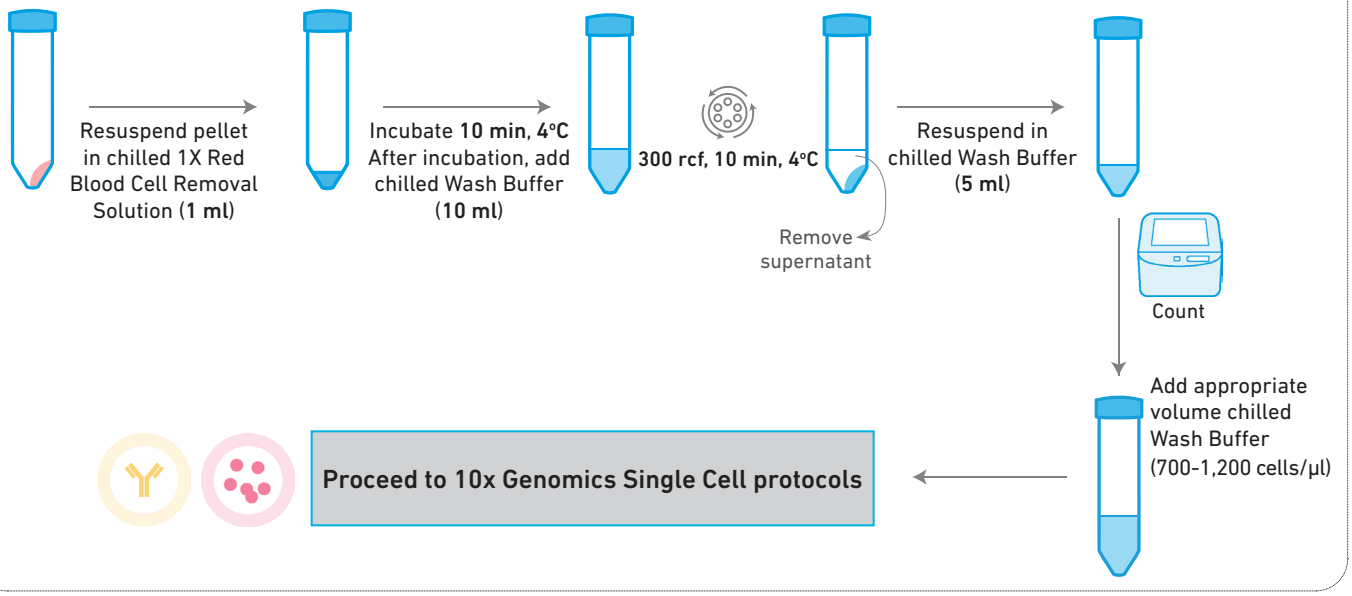
Vendor	Item	Part Number
Thermo Fisher Scientific	Dulbecco's Phosphate-Buffered Saline (DPBS), No Calcium, No Magnesium	14190144
	UltraPure BSA (50 mg/ml)	AM2616
	Nuclease-free Water (not DEPC-treated)	AM9937
	Trypan Blue Stain, 0.4%	T10282
	Countess II FL Automated Cell Counter	AMQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
Miltenyi Biotec	MACS Multistand	130-042-303
	Tumor Dissociation Kit, mouse	130-096-730
	Red Blood Cell Lysis Solution (10X)	130-094-183
	MACS SmartStrainers (70 µm)	130-098-462
	gentleMACS Octo Dissociator with Heaters	130-096-427
	gentleMACS C Tubes	130-093-237
Corning	RPMI 1640	10-040-CV
ATCC	Dulbecco's Modified Eagle Medium (DMEM)	30-2002
Eppendorf	DNA LoBind Tubes, 2.0 ml	022431048

Protocol Overview

1. Tumor Dissociation



2. Red Blood Cell Lysis



Protocol

This protocol was demonstrated using commercially-sourced fresh mouse tumors (300-500 mm^3). The tissues were not frozen.

1. Tumor Dissociation

a. Wash the tumor tissue in a 50-ml centrifuge tube by adding **10 ml** chilled 1X DPBS.

b. Place in a petri dish and cut the tumor tissue to small pieces of ~2-4 mm^3.

c. Prepare enzyme mix in a gentleMACS C Tube by adding:

- **2.35 ml** RPMI 1640 or DMEM
- **100 µl** Enzyme D
- **50 µl** Enzyme R
- **12.5 µl** Enzyme A

d. Transfer the tumor tissue pieces to the C Tube containing the enzyme mix.

e. Tightly close the C Tube and attach upside down to a sleeve of a gentleMACS Octo Dissociator with Heaters. The tissue in the C Tube should be close to the stator of the dissociator.

f. Select the dissociator program based on tumor tissue texture (soft, medium or hard tissue).

g. Run the program 37C_m_TDK_1 for melanoma and colon tumor (soft and medium tissue, respectively). Run the program 37C_m_TDK_2 for breast tumor (hard tissue).

h. At the end of the run, detach the C Tube from the dissociator.

i. Centrifuge at **300 rcf** for **30 sec** at **room temperature.**

j. Remove the supernatant without disturbing the cell pellet.

k. Add **10 ml** RPMI 1640 or DMEM and gently pipette mix to resuspend the cell pellet.

l. Filter the cell suspension through a prewetted 70-µm MACS SmartStrainer placed on a 50-ml centrifuge tube.

m. Wash the strainer with **10 ml** RPMI1640 or DMEM and collect the wash in the tube with the cell suspension.

n. Centrifuge the cell suspension at **300 rcf** for **7 min** at **room temperature**.

o. Remove supernatant without disturbing the cell pellet. Proceed **immediately** to Red Blood Cell Lysis.

2. Red Blood Cell Lysis

The red blood cells present in the dissociated tumor cell suspension are lysed by the Red Blood Cell Removal Solution. Prolonged exposure to this solution could lyse the tumor cells.

a. Add **1 ml** chilled 1X Red Blood Cell Removal Solution to the cell pellet from step 1o and gently pipette mix to resuspend the cells.

DO NOT vortex.

b. Incubate for **10 min** at **4°C**.

c. Add **10 ml** chilled Wash Buffer.

d. Centrifuge at **300 rcf** for **10 min** at **4°C**.

e. Remove supernatant without disturbing the cell pellet.

f. Add **5 ml** chilled Wash Buffer and gently pipette mix to resuspend the cell pellet.

g. Determine the cell concentration and viability using a Countess II FL Automated Cell Counter or hemocytometer. If the percentage of viable cells <70%, follow Demonstrated Protocol Removal of Dead Cells from Single Cell Suspensions for Single Cell RNA Sequencing (Document CG000093). Multiple rounds of dead cell removal may be performed to obtain a high viability sample.

h. Add appropriate volume chilled Wash Buffer to the cell suspension and gently pipette mix to achieve the target cell concentration of 700-1,200 cells/µl.

i. Proceed **immediately** with the 10x Genomics Single Cell protocol.

Results

After tumor dissociation, trypan blue staining of the sample showed a high percentage (68%) of dead cells. To reduce the number of dead cells after tumor dissociation, the protocol outlined in Demonstrated Protocol Removal of Dead Cells for Single Cell RNA Sequencing (Document CG000093) was followed. After dead cell removal, the dead cell fraction was reduced from 68% to 12%, thus increasing the percentage of viable cells in the sample. Results may vary between different tumor types.

Before Dead Cell Removal

After Dead Cell Removal

Troubleshooting

Problem	Possible Solution
High fraction of non-viable cells after tissue dissociation	Optimize dissociation protocol specific to tissue type to enhance sample quality Reduce fraction of dead cells. Refer to Demonstrated Protocol Removal of Dead Cells for Single Cell RNA Sequencing (Document CG000093) Gently handle cell suspensions by following best practices and reduce cell processing times
High fraction of cellular debris in final solution	Add 1-2 additional wash steps Filter cell suspension with the appropriate strainer Sort cells using flow cytometry Clean sample by density centrifugation
<700 cells/µl after final resuspension	Concentrate cell suspension to achieve target concentration of 700-1,200 cells/µl

Contact:
support@10xgenomics.com
10x Genomics
6230 Stoneridge Mall Road
Pleasanton, CA 94588 USA