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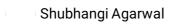
Single cell digestion of tumor tissue

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The purpose of this protocol is to provide details on how to obtain a single-cell suspension from a patient-derived xenograft tumor.

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Single	cell	dige	stion
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Materials	Source	Catalog Number	
DMEM	Gibco	11965092	
Fetal bovine	Global Life	SH3039603	
serum (FBS)	Sciences		
	Solutions		
Type I	Gibco	17100017	
collagenase			
DNase I	Thermo Scientific	89836	
ROCK Inhibitor (Y-27632)	Sigma Aldrich	SCM075	
Gentamicin	Gibco	15750060	
HEPES-buffered saline (HBS)	Gibco	15630080	
Red Cell Lysis Buffer	Roche	11814389001	
Cell Freezing Medium-DMSO 1×	Sigma Aldrich	C6164	
Scissors	Kent Scientific	INS600393	
Scalpels with blade no.10	Fisher Scientific	12-460-451	
Petri dishes	Millipore Sigma	P5481	
15 mL conical tubes	Corning	430055	
50 mL conical tubes	Corning	430829	
70 µm cell strainers	Corning	431751	
40 μm cell strainers	Corning	431750	
Pipettes tips (1000 ul)	Millipore Sigma	AXYT1000B	
Trypan Blue dye	Sigma Aldrich	93595	
Corning CoolCell	Corning	432000	
Cryogenic vial	Nalgene	V5007	

Preparation of Medium

Prepare digestion medium DMEM supplemented with



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- a. [M]10 % volume FBS,
- b. [M]200 units/ml of type I collagenase,
- c. [M]1 units/ml DNase I,
- d. [M]2 Micromolar (µM) Y-27632 and
- e. [M]100 ug/ml Gentamicin
- 2 Prepare storage medium DMEM supplemented with
 - a. [M]10 % volume FBS, and
 - b. [M]100 ug/ml Gentamicin

Harvest Tumor Tissue

- 3 Prepare an ice bucket with ice and 50 ml falcon tubes with 35-45 mL HBS.
- 4 Euthanize the tumor-bearing mouse and extract the tumor.
- 5 Blot the tumor on a Kim-wipe to get rid of excess blood.
- 6 Weigh the tumor and note the weight.
- Place the fresh tumor tissue in a falcon tube containing **35-45 mL** HBS.
- 8 Transfer the falcon tube into a BSL2 hood.

Tissue digestion process (performed in BSL2 hood) 6h 3m

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Prepare a petri dish containing fresh HBS and place it on ice.

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- 10 Transfer the tissue from the falcon tube into the petri dish containing HBS.
- 11 Wash tumor multiple times with **10 mL** HBS and remove excess HBS. Repeat this process two more times.
- 12 Mince the tumor with scissors or scalpels in the petri dish.

For larger tissue (larger than 1 cm³), use multiple rounds of mincing

- 13 Add digestion medium to the petri dish containing tumor tissue and add enough volume to thoroughly cover the entire tumor.
- 14 Place the petri dish in an incubator at **§ 37 °C 95% air/5% CO2** over **© 02:00:00 © 04:00:00** hrs.

Vigorously mix the tissue every 10-15 minutes using a pipette to aid dissociation.

- 15 Periodically assess the digestion under the microscope and stop once small clusters or clumps of cells are released from the tissue and before complete digestion to single cells occurs.
- Transfer the above solution into a 50 ml falcon tube and centrifuge the above solution at **300 x g, 4°C** for 5 mins.
- Discard the supernatant and incubate the digested tissue with **5 mL** Red Cell Lysis Buffer for **00:03:00 mins**.

18	Centrifuge the	above solu	tion at 🚓	300 x g,	4°C	for 5 mins.
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- Discard the supernatant and resuspend the cell pellet in ■50 mL fresh DMEM prepared in step 2.
- Pass the above solution containing tumor cells through a 70 um filter and collect the solution containing tumor cells that passed through the filter in a fresh 50 ml falcon tube.
- Pass the above solution containing tumor cells through a 40 um filter and collect the cells that passed through the filter in a fresh 50 ml falcon tube.
- 22 Centrifuge the solution containing tumor cells at **300 x g, 4°C** for 5 mins.
- 23 Discard the supernatant and resuspend the cell pellet in fresh DMEM prepared in step 2.
- 24 Perform Trypan Blue dye exclusion test and note the live and total cell count.
- Prepare cryogenic vials with □900 μL of □1 M live cells and □100 μL of DMSO per cryovial.
- 26 Place the prepared cryovials in a freezing container and place the container in 8 -80 °C freezer for 24 hours.
- 27 After 24 hours, transfer the cryovials into liquid nitrogen storage for long-term storage.