Celinus 서비스 의뢰 시 샘플 준비 가이드

1. Sample preparation at a glance (p.1)

For single cell transcriptome analysis					
Blood		≥ 5 ml		Drawn directly into CPT* tubes *Caution! Fragile!	25 ℃ (up to 2h) 4 ℃ (over 2h)
PBMC	Fresh PBMC	Cells condition : ≥ $5x10^5$ cells/vial	Viability ≥ 80% (Total cell 기준)	Media or 1X PBS Suspension	4 ℃ (ice pack X, ice O)
	Frozen PBMC	Cells condition : ≥ $1x10^6$ cells/vial	Viability ≥ 80% (Total cell 기준)	(Recmd) Cell Banker 1ml or 10% DMSO+40% FBS +Media	−80 ℃ (dry ice O)
Tissue	Fresh Tissue	Stored in 15 ml or 50 ml tube		Media or 1X PBS or Storage Sol.	4 ℃ (ice pack X, ice O)
	Fresh cell from tissue	Cells condition : ≥ $5x10^5$ cells/vial	Viability ≥ 80% (Total cell 기준)	Media or 1X PBS Suspension	4 ℃ (ice pack X, ice O)
	Frozen cell from tissue	Cells condition : ≥ $1x10^6$ cells/vial	Viability ≥ 80% (Total cell 기준)	(Recmd) Cell Banker 1ml or 10% DMSO+40% FBS +Media	−80 ℃ (dry ice O)

2. Sample preparation protocol for Single cell transcriptome analysis (p.2 ~ 4)

2. Sample preparation protocol for single cell transcriptome analysis

The protocol is to prepare single cell suspensions for single cell RNA sequencing.

The protocol is based on using Miltenyi tumor dissociation kit to dissociate the tissue sample to prepare frozen cell stock.

- All steps should be performed under sterile conditions.
- The reagents and the incubation time may vary depend on the tissue type.
- Tumor Dissociation Kit as well as Ficoll could be substituted depend on sample species (Human, Mouse etc.)

1. Materials

Please regard the following as recommendations except the reagent for cell stock solution.

Category	Product	Manufacturer	Cat #
Pipet & Tip	10 μl Tip	Vertex	#4137NS0
	200 μl Tip	Vertex	#4237NS0
	1000 μl Filtered Tip	Vertex	#4337NSF
	1000 μl Wide Bore Tip	Rainin	#30389218
	10 ml Pipet	Thermo	#170356N
	25 ml Pipet	Thermo	#170357N
Tube	gentleMACS C tube	Miltenyi Biotec	#130-093-237
	1.5 ml tube	Eppendorf	#0030 180.051
	15 ml tube	Corning	#430791
	50 ml Tube	Corning	#430829
	2 ml Cryotube	Nunc	#368632
Reagents	Ficoll 100 ml	GE Healthcare	#17-1440-02
	CELLBANKER 1 100 ml/ 10% DMSO + 40% FBS + Culture Media / Mr. Frosty Freezing Container/ Isopropanol/LN2 Tank	ZENOAQ	#BLC-1
	RPMI 1640 or DMEM 500 ml	Gibco	#22400-089 #11995-065
	1X PBS 500 ml	Gibco	#10010023
Others	60 mm Petri dish	Thermo	#150462
	10 ml Syringe	Koreavaccine	#KOVAC-SYRINGE 10ml
	70 μm Strainer	Falcon	#352350
	Sterile scissors, Sterile forceps		
	Pipet aid	Drummond	
	20, 200, 1000 pipet	Rainin	
	Cell counting chamber slides set (Including trypan blue (0.4%))	Thermo	#C100228
	70% EtOH		
Instrument	MACSmix™ Tube Rotator	Miltenyi Biotec	#130-090-753
	gentleMACS Dissociator 37˚C Incubator or CO2 Incubator or gentleMACS Octo Dissociator with Heaters	Miltenyi Biotec	#130-093-235 #130-096-427
	Countess™ II FL Automated Cell Counter	Thermo	#AMQAF1000
	Deep freezer		
	Digital scale	Innotem	
Kit	Tumor Dissociation Kit, human	Miltenyi Biotec	#130-095-929

2. Methods

2.1 Sample Collection

A. Cell Collection from Tissue

(1) Prepare C tubes as much as the tissue sample

(2) Dissociate the tissue sample using gentleMACS Dissociator + incubator at 37 ˚C (or gentleMACS Octo Dissociator with Heaters) and Tumor dissociation kit (refer to the ref 'gentleMACS')

(3) Place 70 μm Strainer on a 50 ml tube and wash the Strainer with 2 ml RPMI 1640 or DMEM. Apply the dissociated cell suspension to 70 μm Strainer *If Strainer is clogged, change it to new one*

(4) Centrifuge the filtered cell suspension and aspirate the supernatant completely

(5) Resuspend sample with RPMI 1640 or DMEM

(6) Remove RBC using Ficoll or RBC lysis buffer (refer to the ref 'Isolation of mononuclear cells or RBC Lysis Buffer Protocol')

(7) Wash the separated cells with PBS (volume of PBS can be changed depend on the pellet size) then centrifuge

(8) Resuspend the pellet with media or PBS (volume of PBS can be changed depend on the pellet size)

(9) Count cells *Take a cell counting method either auto cell counter or hemocytometer counting*

B. PBMC collection from Blood

(1) Dilute cells with 2–4 X the volume of PBS

(2) Carefully layer 35 ml of diluted cell suspension over 15 ml of Ficoll in a 50 ml conical tube

(3) Centrifuge at 400 x *g* for 30–40 minutes at 20 ℃ in a swinging–bucket rotor without brake (decal: 0)

(4) Aspirate the upper layer leaving the mononuclear cell layer (lymphocytes, monocytes, and thrombocytes) undisturbed at the interphase

(5) Carefully transfer the mononuclear cell layer to a new 50 ml conical tube

(6) Fill the conical tube with media and centrifuge at 300 x *g* for 5 minutes at 20 ℃. Carefully remove supernatant completely

(7) For removal of supernatant, resuspend the cell pellet in 15 ml of Media and centrifuge at 300 x g for 5 minutes at 20 ℃. Carefully remove the supernatant completely (This step will increase the purity of the target cells in the subsequent MACS Cell Separation)

(8) Repeat step 7 (Optional: Most of the platelets will remain in the supernatant upon centrifugation at 200 x *g*)

(9) Count cells counting *Take a cell counting method either auto cell counter or hemocytometer counting*

C. Frozen Tissue (Needle biopsy)

(1) Freeze the tissue in a cryotube or transportable container

(2) Put it in dry ice and be careful not to melt the tissue

D. Cell Stock

Cell stock concentration	Not less than 1x10^6 cells/ml per 1 vial * 2 vials of cell stock are needed
Cell viability	Above 80% (total cell)

*Sample availability will be determined by cell loss ad cell viability after thawing

(1) Concentration of the cell stock should be determined based on the cell counting result

(2) Take out the sample to a new tube as much needed for cell stock or proceed to the next step without aliquot

(3) Centrifuge at 4 ˚C, remove the supernatant

(4) Stock the pallet at 2 ml cryotube following either of below

a. Cellbanker 1

① Resuspend cell pellet with 1 ml cellbanker

② Dispense the cell suspension in 2 ml cryotube

③ Place the vial directly in a − 80 ˚C for storage

b. 10 % DMSO + 40 % FBS + Culture Media (fill isopropanol in Mr. Frosty Freezing Container)

① Resuspend cell pellet with 1 ml solution (10% DMSO + 40% FBS + Culture Media)

② Dispense the cell suspension in 2 ml cryotube

③ Place the vial in the container

④ Place the container in a − 80 ˚C for overnight

⑤ Transfer the frozen vials to liquid nitrogen storage tank

2.2 Send the frozen cell stock vial or Frozen Tissue to GENINUS on dry ice package

2.3 Reference

1. Miltenyi Biotec, Tumor Dissociation Kit, human https://www.miltenyibiotec.com/US-en/products/macs-sample-preparation/tissue-dis sociation-kits/tumor-dissociation-kit-human.html
2. Miltenyi Biotec, gentleMACS Dissociator Manual https://www.miltenyibiotec.com/_Resources/Persistent/57168e4672fe7db169aaa01b45dbf89e83e2786c/gentleMA CS_Dissociator_user_manual.pdf
3. Miltenyi Biotec, gentleMACS™ Octo Dissociator with Heaters Manual https:// www.miltenyibiotec.com/KR-en/products/macs-sample-preparation/tissue-dissociato rs-and-tubes/gentlemacs-octo-dissociator-with-heaters/gentlemacstm-octo-dissociator -with-heaters.html#130-096-427
4. Miltenyi Biotec, MACSmix™ Tube Rotator Manual: https://www.miltenyibiotec.com/KR-en/products/macs-sample-preparation/sample-m ixing/macsmixtm-tube-rotator.html#130-090-753
5. GE Healthcare, Isolation of mononuclear cells: https://www.sigmaaldrich.com/co ntent/dam/sigma-aldrich/docs/Sigma-Aldrich/General_Information/1/ge-isolation-ofmononuclear-cells.pdf
6. Intronbio, G-DEX™ IIb RBC Lysis Buffer Protocol https://www.intronbio.co.kr:6002/intronbio/product/product_view.php?PRDT_ID=18 #none
7. 10x genomics recommended websites for Tissue Dissociation information: 1) Miltenyi Biotec 2) Worthington Biochemical 3) Sigma-Aldrich 4) Roche Diagnostics
8. Protocol by Tissue Type: http://www.worthington-biochem.com/tissuedissociation/default.html