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Non-Enzymatic Generation of Placenta Single Cells from Third Trimester Human Placenta

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ABSTRACT

The placenta is a heterogeneous and complex organ with multiple cell types, posing a challenge for the field of maternal-fetal medicine to implement single-cell technologies for a deeper characterization of this essential organ. Several protocols use enzymes to digest the tissue and generate single cell suspension, but this approach has several shortcomings including the loss and reduced viability of cells. In this study, we describe a non-enzymatic approach to generate single cell suspension
from placental tissue with high yield and viability for single cell RNA sequencing.

ATTACHMENTS

non-enzymatic single cell generation edited.pdf

MATERIALS

S/N	EQUIPMENT/CONSUMMABLES	COMPANY
1.	Styrofoam box with insulated container	Uline

Protocol status: Working
We use this protocol and it's working

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	2.	1.5ml microcentrifuge tubes	Eppendorf
	3.	Ice making machine	Kojak
	4.	Weighing Balance	Halomedicals Systems Limited
	5.	Gentle MACs Dissociator	Miltenyi Biotech
	6.	Gentle MACs C- tubes	Miltenyi Biotech
	7.	Countess III Chamber slides	Thermo Fisher Scientific
	8.	Countess III Automated Cell counter	Thermo Fisher Scientific
	9.	100-1000µl Micro pipette	Agros 240-21 Omega 8
		0.1-10µl Micro pipette	Agros 240-21 Omega 8
	10.	Refrigerated Micro Centrifuge	Eppendorf
	11.	100µl Pippete tips	Argos technologis
	12.	10µl Pippete tips	Argos technologis
	13.	1000µl Pippete tips	

Placenta Single Cell Preparation Protocol

10m 30s

1 Transform placenta sections into gentle MACs C-tube containing 3000 µL of MACs running buffer.

2 Set up a gentle MACS dissociator program to h-cord-01 for 553 rotation per round (rpr) at 00:00:30 . 30s

3 After the run, centrifuge at 300 x g for 00:10:00 at 40 °C . 10m

4 Remove the supernatant leaving 200 µL to resuspend pellet in. Transfer pipettes may be used to remove supernatant after centrifugation to minimize loss of placenta cells.

5 10 µL of placental cells can be counted on a countess II cell counter or using trypan blue.