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

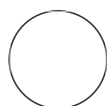
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### ABSTRACT

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

**Created:** Dec 13, 2023

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92360

**Funders**

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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


30s

 00:00:30 .


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.

