



Dec 27, 2023

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

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvm3b99l3p/v1>

Ameloko Joy^{1,2}, Idowu Aimola^{1,2}, Fomukong A Hanneda^{1,2}, Reuben B. Samson^{1,2}, Zeenat B Kudan^{1,2}, Habiba H. Abubakar³, Musa Kana³, Benedict Anchang⁴

¹Africa Center of Excellence for Neglected Tropical Diseases and Forensic Biotechnology, Ahmadu Bello University, Zaria Nigeria;

²Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria;

³Kaduna Infant Development Birth Cohort Project Kaduna, Nigeria;

⁴National Institute of Environmental Health Sciences, Raleigh, North Carolina



Idowu Aimola

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Protocol Citation: Ameloko Joy, Idowu Aimola, Fomukong A Hanneda, Reuben B. Samson, Zeenat B Kudan, Habiba H. Abubakar, Musa Kana, Benedict Anchang 2023. Non-Enzymatic Generation of Placenta Single Cells from Third Trimester Human Placenta. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.yxmvm3b99l3p/v1>

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Protocol status: Working

We use this protocol and it's working

Created: December 13, 2023

Last Modified: December 27, 2023

Protocol Integer ID: 92360

Keywords: placenta single cell, single cell rna, viability for single cell rna, placental tissue with high yield, third trimester human placenta the placenta, placental tissue, third trimester human placenta, placenta, single cell, single cell suspension, fetal medicine, rna, multiple cell type, reduced viability of cell, complex organ with multiple cell type, cell, cell technology, sequencing, enzyme

Funders Acknowledgements:

Chan Zuckerberg Initiative

Grant ID: DAF2022-240134

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...](#)

203KB

Materials

	S/ N	EQUIPMENT/CONSUMMABLES	COMPANY
	1.	Styrofoam box with insulated container	Uline
	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 Pipette tips	Argos technologis
	12.	10µl Pipette tips	Argos technologis
	13.	1000µl Pipette 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 . 
- 3 After the run, centrifuge at  300 x g for  00:10:00 at  40 $^{\circ}\text{C}$.  
- 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. 