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Enzymatic disaggregation of human myometrium for 10X Single Cell RNA-seq

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1 Works for me dx.doi.org/10.17504/protocols.io.bb5miq46

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ABSTRACT

This protocol describes the procedure for dissociating myometrial samples, which is based in an enzymatic disaggregation using Collagenase IV and DNAse I. This protocol is adapted from Mas et al. 2012 *Fertil Steril* with minor modifications.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Mas A, et al. Identification and characterization of the human leiomyoma side population as putative tumor-initiating cells. Fertil Steril. 2012 Sep;98(3):741-751.

GUIDELINES

Storage conditions of reagents

Reagent	Storage condition
Hypothermosol	4ºC
HBSS	4ºC
HEPES 1M	4ºC
DNAse type I	Resuspend in PBS and store 100 µl aliquots at -20ºC
Collagenase type IV	4ºC
DMEM low glucose	4ºC
FBS	Store 30 ml aliquots at -20ºC
Penicillin-Streptomycin (10000 U/ml)	Store 5 ml aliquots at -20ºC

Required equipment

Equipment	Supplier	Catalog nº
Heracell 150i CO2 incubator	Thermo Scientific	51026280
New Brunswick Incubator shaker Innova 40	Eppendorf	M1299-0092
Centrifuge Mega Star 600	VWR collection	521-1893
Microscope Nikon eclipse TE200	Nikon	-
Class II microbiological safety cabinet MSC advantage	Thermo Scientific	51025411

The protocol workflow is as follows:

1. Tissue collection and cold transportation until processing
2. Dissociation: mechanic and enzymatic
3. Remove red blood cells if necessary
4. Prepare cells for Chromium

MATERIALS

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NAME	CATALOG #	VENDOR
BRAND® counting chamber BLAUBRAND® Neubauer improved New without clips, double ruled	BR717805	Sigma
Penicillin-Streptomycin	15140122	Gibco - Thermo Fisher
Collagenase Type 4	LS004188	Worthington Biochemical Corporation
100 µm Cell Strainer	352360	Falcon
HypoThermosol® FRS Preservation Solution	H4416	Sigma Aldrich
TrypLE™ Select Enzyme (1X), no phenol red	12563029	Thermo Fisher
HEPES (1 M)	15630080	Thermo Fisher
Nalgene™ Rapid-Flow™ Sterile Disposable Filter Units with PES Membrane, 250mL, 0.2µm pore, 50mm membrane	568-0020	Thermo Fisher
ACK Lysing Buffer	A1049201	Thermo Fisher
HBSS (1x)	14175-095	Gibco - Thermo Fisher
Deoxyribonuclease I from bovine pancreas	D4513	Sigma-aldrich
Dulbecco's Modified Eagle's Medium - low glucose	D5921	Sigma-aldrich
Fetal bovine serum (FBS)	S181B-500	BioWest
Falcon 40 µm Cell Strainer	352340	Corning
Sterile surgical blades	7806607	Aesculap

SAFETY WARNINGS

Human samples including tissue, blood and bodily fluids have the potential to harbour HG2 and Hazard Group 3 (HG3) organisms, specifically Blood Borne Viruses (BBVs). As security warning, please follow the procedures related to Biological Safety at Containment Level 2.

BEFORE STARTING

Prepare Wash buffer:

Make stock of wash buffer and store at 4 °C. Aliquot 49.5 mL of HBSS in a 50 ml conical Falcon tube and add 500 µl of Pen/Strep (100 U/ml).

Prepare Enzyme buffer:

To make 100 ml of enzyme buffer, combine 95.6 ml of HBSS with 1 ml of Pen/Strep (100 U/ml); 1 ml of HEPES (1M); 4.6 ml of wash buffer; 95 µl of DNAse and 135 mg of collagenase IV and filter in a 0.2 µm bottle-top sterile filter unit.

Prepare resuspension buffer:

Add 12 ml of inactivated FBS and 1 ml of Pen/Strep (10000 U/ml) to 87 ml of DMEM medium (low glucose) to prepare 100 ml of resuspension buffer.

Tissue collection and transportation

1 Uterine tissues should be transported in preservation solution (HypoThermosol® FRS) at 4 °C from the surgery room to the lab.

Dissociation

2 Rinse the sample with wash buffer, removing blood or mucus.

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3 Place wet tissue under a petri dish. Isolate with a sterile scalpel the myometrial layer by removing the endometrium, serosa and laser-burnt zones.

4 Mechanical disaggregation by chopping and thoroughly mincing up the tissue into small pieces (<1 mm^3).

5 Transfer contents to 50 ml falcon tubes containing **30 ml** of enzyme buffer. Tighten lid, seal with parafilm and incubate at **37 °C** overnight horizontally.

6 Afterwards, cell suspensions should be filtered through 100 to 50-µm polyethylene filters to remove cellular clumps and undigested tissue.

7 Centrifuge the filtered medium **400 x g 00:05:00** . Remove supernatant and add at least **1 ml** of resuspension buffer (depending of the pellet content).

Remove red blood cells

8 [Optional] If after centrifugation there is a ring of red blood cells, add ACKL lysis buffer, consisting in hypotonic shock, and incubate at **37 °C** for **00:05:00** . Then, follow step 7 again.

Dissociaton

9 Add **400 µl** Tryple Select Enzyme to resuspended media containing cells and mix thoroughly by pipetting. Incubate for **00:10:00** - **00:15:00** at **37 °C** and centrifuge at **300 rpm** .

10 Add **100 µl** DNAse I to digest the extracellular genomic DNA and mix thoroughly by pipetting. Incubate for **00:05:00** at **Room temperature** .

11 Add at least **1 ml** of resuspension buffer. Filter through a 40 µm cell strainer and centrifuge at **400 x g 00:05:00** .

12 Remove supernatant and resuspend the content in at least **1 ml** of resuspension buffer (depending of the pellet content).

Prepare cells for chromium

13 Count the total number of cells (at least twice on two different cell counters).

14 Proceed to 10x experiment.

15 [Optional] Resuspend cells in freezing medium to a concentration of 1 x 10^6 cells for storage in cryogenic vials.

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