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

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Manuscript citation:
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.

Protocol status: Working
Working. We and other researchers are using and published the results using this protocol (Mas et al., 2015; Prusinski et al., 2018; Corachán et al., 2019).

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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.

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 -20º C
	Collagenase type IV	4ºC
	DMEM low glucose	4ºC
	FBS	Store 30 ml aliquots at -20º C
	Penicillin-Stre	Store 5 ml aliqu

	pto myci n (100 00 U/ml)	ots at -20° C

Required equipment

	Equi pme nt	Sup plier	Cata log n°
	Hera cell 150i CO2 incu bato r	Ther mo Scie ntific	5102 628 0
	New Brun swic k Incu bato r shak er Inno va 40	Epp end orf	M12 99- 009 2
	Cent rifug e Meg a Star 600	VWR colle ction	521- 1893
	Micr osco pe Niko n eclip se TE2 00	Niko n	-
	Clas s II micr obiol ogic al safe	Ther mo Scie ntific	5102 5411

	ty cabinet MSC advantage		

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

MATERIALS

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

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 start

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.

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 1×10^6 cells for storage in cryogenic vials.