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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).

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

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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
Hypotermosol	4°C
HBSS	4°C
HEPESS 1M	4°C
DNase type I	Resuspend in PBS and store 100 µl aliquots at -20°C
Collagenase type IV	4°C
DME M low glucose	4°C

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FBS	Store 30 ml aliquo ts at - 20°C
Penicillin-Streptomycin (1000 U/ml)	Store 5 ml aliquo ts at - 20°C

Required equipment

Equip ment	Suppli er	Catal og n°
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 micro biological 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

MATERIALS

BRAND® counting chamber BLAUBRAND® Neubauer improved New without clip double ruled Sigma Catalog #BR717805
Penicillin-Streptomycin Gibco - Thermo Fisher Catalog #15140122
Collagenase Type 4 Worthington Biochemical Corporation Catalog #LS004188
100 µm Cell Strainer Falcon Catalog #352360
HypoThermosol® FRS Preservation Solution 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, 250m 0.2µm pore, 50mm membrane Thermo Fisher Catalog #568-0020
ACK Lysing Buffer Thermo Fisher Catalog #A1049201
HBSS (1x) Gibco - Thermo Fisher Catalog #14175-095
Deoxyribonuclease I from bovine pancreas Sigma-aldrich Catalog #D4513
Dulbecco's Modified Eagle's Medium - low glucose 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 INSTRUCTIONS

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 mm³).
- 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.

Dissociation

- 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.