Endometrium dissociation with trypsin

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1 Works for me

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ABSTRACT

This protocol is for enrichment of epithelial glands on endometrium; following the step from "Endometrium dissociation with collagenase".

GUIDELINES

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,); and for brain tissue, CNS tissue and CSF, prions. In the UK we can work with such samples at CL2 on the condition that we do not intend to culture any of the organisms that might be contained in the samples and that the samples haven't already been identified by tests or diagnosis as containing HG3 organisms.

MATERIALS

NAME	CATALOG #	VENDOR
10% FBS RPMI		
FBS		Invitrogen - Thermo Fisher
RPMI 1640 Medium	11875093	Thermo Fisher Scientific
Trypsin-EDTA (0.25%) phenol red	25200072	Thermo Fisher Scientific
PBS	View	Invitrogen - Thermo Fisher
DNAse I	4716728001	Sigma

SAFETY WARNINGS

Samples are unscreened human tissues, please adhere to Biological Safety at Containment Level 2 work procedures.

1 Prepare Trypsin-EDTA-DNaseI mix:

Product	Stock	Final volume (10 ml)	Concentration
Trypsin-EDTA (0.25%) phenol red		9.9 ml	
DNaseI	10mg/ml	100 ul	0.1 mg/ml

2 Following step 10 from "Endometrium dissociation with collagenase" protocol, where pieces of tissue are retained on the cell strainer.

Collect pieces retained on the filter by inverting the filter into a new 50 mL tub and adding 45 ml of PBS with a 1 mL pipette.

3 Centrifuge at 450 g for **00:05:00** together with collagenased sample. Discard supernatants.

4 Resuspend the pieces with **10 ml** Trypsin/EDTA 0.25% and incubate for **00:20:00** at **37 °C** while shaking.

5 Add **20 ml** of RPMI with 10% FBS.

6 Filter material slowly and carefully (very dens liquid) through a 100 µM strainer. Discard retained tissue.

7 Centrifuge at 500 rcf for **00:05:00** at **4 °C** . Discard supernatant.

Same step as 14 in "Endometrium dissociation with collagenase" protocol

8 {optional} Resuspend sample with **5 ml** of RLB mix and incubate for **00:10:00**

*RLB preparation: Dilute 10x RLB stock with water

After RBC lysis, add **10 ml** of PBS and centrifuge at 500 rcf for **00:05:00**

9 Resuspend cells in **1 ml** of PBS in an Eppendorf.

10 Centrifuge at 500 rcf for **00:05:00** at **4 °C** .

Discard supernatant.

11 Resuspend with **250 µl** of PBS (final volume depending on cell number)

12 Filter cells trough a cell strainer snap cap tube.

13 Count live cells using tryphan blue and haematocytometer.

14 {Optional} Resuspend cells in freezing medium to a concentration of 1x 10^7 cells and aliquot into cryogenic storage vials.