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Human embryonic gonad dissociation with Trypsin-EDTA

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1 Works for me dx.doi.org/10.17504/protocols.io.66fhhbn

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol is for enrichment of fetal gonadal cells

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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,). 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.

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MATERIALS TEXT

MATERIALS

Gibco Penicillin-Streptomycin (10,000 U/mL) (Pen/Strep) **Fisher Scientific Catalog # 15-140-122**

FBS **Invitrogen - Thermo Fisher**

PBS **Invitrogen - Thermo Fisher**

RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**

Trypsin-EDTA (0.25%) phenol red **Thermo Fisher Scientific Catalog #25200072**

DNaseI **Contributed by users**

SAFETY WARNINGS

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

1 Prepare digestion mix

A	B	C
Reagents	Volume	Concentration (stock)
Trypsin-EDTA (0.25%) phenol red	4ml (depending on tissue size)	0.25% (1X)
DNaseI (0.1mg/ml)	40ul	10mg/ml

Prepare complete media:

A	B	C
Reagents		Final conc.
RPMI	445ml	
FBS	50ml	10%
Pen-strep	5ml	1%

Prepare final resuspension buffer:

A	B	C
Reagents	Volume	Concentration (stock)
PBS	5ml	
BSA 0.04%	20ul	10%

2 Samples arrive in hypothermosol solution.

The gonad is washed with PBS and dissociated into single-cells following the protocol below.

3 Fragment the gonads with a scalpel into 2 to 20 pieces per gonad (depending on developmental stage).

4 Suspend gonadal fragments into a 15 mL tube containing **4 mL** of Trypsin-EDTA (0.25%) + DNaseI mix. Volume is

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depending on tissue size.

For small tissues: **750 µl** in an eppendorff

5 Place the tube/s into the incubator at **37 °C** for **00:15:00** . Rotate and shake to make sure that the gonadal fragments are uniformly distributed on the wall of the tube.

For small tissues: incubate epp at **37 °C** : 5 min incubation + up & down pipetting + 5 min incubation + up & down pipetting + 5 min incubation

6 If there are still fragments of tissue in suspension, incubate for 5-10 more minutes. Otherwise continue to next step. This will avoid unnecessary damage on the already dissociated cells. Take a note if this is the case.

7 Add **9 mL** of complete medium with pen-step. Pipette up and down.

For small tissues: add **500 µl** up & down pipetting

8 Filter suspension with a 100 uM filter in 50 ml tube. Top it up with complete medium up 50 ml.

For small tissues: Filter suspension in a FACs tub strainer and then transfer it to an eppendorf

9 Centrifuge at 500xg for **00:05:00** at **4 °C**

10 Discard the supernatant and suspend the cellular pellet in **1 mL** of PBS. Transfer to 1.5 ml eppendorf tube.

11 OPTIONAL *If pellet is reddish, add RBC lysis buffer and incubate for 10 min at room temperature.
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*RBC lysis buffer preparation: Dilute 10X RBC lysis buffer stock with water.

After RBC lysis, add 1 ml of PBS and centrifuge 500 g for 5 min.

Wash once with PBS 0.04% BSA.

12 Centrifuge at 500xg for **00:05:00** at **4 °C**

13 Discard supernatant and re-suspend in 250 ul PBS 0.04% BSA. Adjust volume accordingly depending on cell count.

For small tissues: re-suspend in 100 ul PBS 0.04% BSA

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