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Human embryonic gonad dissociation with Collagenase & Trypsin V.1

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1 *Works for me* dx.doi.org/10.17504/protocols.io.br4gm8tw

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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,); 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 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**

DNaseI **Contributed by users**

Collagenase IA by Sigma Aldrich Catalog # C2674-100MG
Liberase™ TM Research Grade by Sigma Aldrich Catalog # 5401119001

SAFETY WARNINGS

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

Prepare collagenase mix

1

Heat-inactivate FBS at **56 °C** for **00:30:00** before use.

Collagenase mix recipe:

Product	Stock	Final volume (10 ml)	Concentration
RPMI or Hams F12 + 10% FBS	9 ml RPMI or Hams F12 + 1ml FBS	8.8 ml	
Collagenase IA	10 mg/ml	1 ml	1 mg/ml
Liberase TM	5 mg/ml	100 ul	50 ug/ml
DNase I	10 mg/ml	100 ul	0.1 mg/ml

2 **Digestion mix recipe:**

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

Tissue dissociation and digestion 20m

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3 Wash tissue with PBS.

4 Place wet tissue under a petri dish. Take 2 scalpels and roughly mince up the tissue. This step is crucial to increase the efficiency of the digestion.

5 Transfer contents to 15ml falcon containing the collagenase mix (~ **10 mL** /tissue but it will depend on the size of the tissue.

6 Tighten lid and then seal with parafilm.

7 Incubate at **37 °C** for **00:45:00** . Shacking every 10 min during the incubation is recommended.

8 Filter sample through small strain (100 um) and keep the filter.

9 Centrifuge 450 g, **00:05:00** (0.5 rcf, 5mins).

10 Remove carefully 90% of the media by decanting the tube (leave between 0.5 ml and 1 ml).

11 Resuspend the cell pellet in 2 ml of PBS and pass it through the inverted filter adding extra 3 mL of PBS

12 5m

13 Remove carefully 90% of the media by decanting the tube (leave between 0.5 ml and 1 ml) and transfer to a 1mL eppendorf

14 5m
Centrifuge 450 g, **00:05:00** (0.5 rcf, 5mins) and discard supernatant

15 Add 700 microL Trypsin-EDTA/DNase I and Incubate at **37 °C** for 10 min with rotation.

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16 Add 700 microL RPMI complete and filter trhough a Facs tube passing 1mL more of media.

17 Transfer to a 5 mL eppendorf and Centrifuge 450 g, **00:05:00** (0.5 rcf, 5mins) 5m

18 Discard supernatant without disrupting the cell pellet and with 1 mL of PBS-BSA 0.04%, transfer to a 1,5 mL eppendorf

19 Centrifuge 450 g, **00:05:00** (0.5 rcf, 5mins) and leave 200-300 microL 5m

20 Proceed to cell count with disposable C-chip haematocytometer.

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