Mar 23, 2021

Human embryonic gonad dissociation with Collagenase IV

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1 Works for me dx.doi.org/10.17504/protocols.io.btjznkp6

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SUBMIT TO PLOS ONE

ABSTRACT

This protocol is for enrichment of fetal gonadal cells

DOI

dx.doi.org/10.17504/protocols.io.btjznkp6

PROTOCOL CITATION

Carmen Sancho, Regina Hoo, Roser Vento-Tormo 2021. Human embryonic gonad dissociation with Collagenase IV. **protocols.io** https://dx.doi.org/10.17504/protocols.io.btjznkp6

CREATED

Mar 23, 2021

LAST MODIFIED

Mar 23, 2021

PROTOCOL INTEGER ID

48473

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, Type IV, powder CAT# 17104019
DNAse I bySigma CAT# 4716728001
100 µm Cell Strainer byFalcon CAT#352360
LiberaseTM by Roche CAT# 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:

A	B	C	D
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 IV	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

Tissue dissociation and digestion

2 Wash tissue with PBS.

3 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.

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

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5 Tighten lid and then seal with parafilm.

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

7 Filter sample through small strain (100 um) and discard the filter.

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

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

10 Resuspend the cell pellet in 5 ml of PBS. Centrifuge 450 g for 5min. Discard supernatant.

11 Resuspend cells with 500microL of PBS-BSA. Proceed to cell count with disposable C-chip haematocytometer.

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