



Preparing single-cell suspension from human embryo/fetus

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[dx.doi.org/10.17504/protocols.io.xmbfk2n](https://doi.org/10.17504/protocols.io.xmbfk2n)

Human Cell Atlas Method Development Community

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ABSTRACT

Protocol for human embryo/fetal tissue handling and dissociation, suitable for subsequent single-cell RNA sequencing and in situ hybridization.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

- This protocol has mainly been used for dissociating human embryonic brain tissue at postconceptional week (PCW) between 4 to 10. It has also been adapted for human embryonic heart dissociation using Collagenase type II instead of Papain.
- Key to a viable cell suspension is to work quickly, in ice cold media (except where noted) and with well-oxygenated solutions.
- Incubation times may vary depending on the region (or tissue) and age being used and have to be tested. However, the incubation time with Papain should be kept on the short side when working with younger time points, the tissue should easily fall apart when starting the trituration.

MATERIALS

NAME ▾	CATALOG # ▾	VENDOR ▾
1.5 mL Eppendorf tubes		
15 mL Falcon tubes		
Hemocytometer (Neubauer)		
Papain Dissociation System (PDS kit)	LK003150	Worthington Biochemical Corporation
Collagenase Type 2	LS004174	Worthington Biochemical Corporation
Earls Balanced Salt Solution (EBSS) PDS kit	LK003188	Worthington Biochemical Corporation
Bovine Serum Albumin solution 30% in DPBS	A9576	Sigma Aldrich
CellTrics 30 um filter	04-0042-2316	
Petri dish 35 mm	View	

SAFETY WARNINGS

Consult local regulations for working with human fetal tissue. It is recommended to test for infectious agents (e.g. hepatitis B and C, HIV, syphilis and HTLV) and to work in a BSL2 laboratory with suitable splash protection (e.g. protective goggles and surgical mask, and/or a plexiglas splash protection screen). However, local regulations and requirements will vary by country, institution and over time, and you should make sure you are following all necessary regulations for your safety.

- Prepare fire polished Pasteur pipettes with different diameters of the tip suitable for the dissociation.

Preparations

- 1 Oxygenate Earl's Balanced Salt Solution (EBSS) by bubbling with 95% O₂:5% CO₂ on ice for **5-10 min** (until appropriate pH is reached).
- 2
 - Add 5 mL EBSS to Papain vial, dissolve at **37 °C** , then move to room temperature (RT).
 - Add 500 µL EBSS to DNase vial, keep at RT. Once dissolved, add 250 µL DNase to the Papain vial.



For embryonic/foetal heart dissociation, 5 mL of collagenase II is used with a concentration of 200 U/mL instead of Papain.

- 3 Start pre-cooling the centrifuge at 4-8 °C while dissolving the enzymes. This is a good time to take images of the tissue and prepare the dissection instruments used for cutting the tissue.

Dissociation

- 4 Cut the tissue into smaller pieces and transfer the pieces to the Papain vial using a pair of tweezers.
- 5 Incubate the tissue for **25-40 min** at **37 °C** . Incubation time may vary depending on age and region. Younger tissue usually requires shorter time.



For embryonic/foetal heart dissociation, the incubation time varies between 40 min - 1 h or more.

- 6
 - After incubation, carefully triturate the tissue until pieces fall apart by using a fire polished Pasteur pipette.
 - If undissociated pieces are still present, continue incubation 2-5 min more and triturate again (pieces should fall apart easily).
- 7
 - Filter the dissociated cells through an EBSS-equilibrated cell strainer of Partec filter.
 - Spin the cells at **200 g** for **5 min** at 4-8 °C.
 - (If needed, perform gradient centrifugation: resuspend the pellet in **900 µL EBSS**, **100 µL Albumin inhibitor**, **50 µL DNase** and carefully layer on top of **3 mL Albumin inhibitor** solution. Centrifuge at 70 g for 6 min at 4-8 °C with slow acceleration and deceleration). This step can be used to remove blood cells from cell suspensions of the heart.
- 8
 - Remove the supernatant and resuspend the pellet in approximately 100 µL (or more if too concentrated) with a fire polished Pasteur pipette.
 - Transfer the cell suspension to a 1.5 mL Eppendorf tube pre-coated with 30% bovine serum albumin (BSA).
 - Count the cells using a hemocytometer or an automated cell counter. Adjust the concentration as desired for the 10X Chromium platform.



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