DEMONSTRATED PROTOCOL

Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing

Overview

This protocol outlines how to obtain a single cell suspension from embryonic mouse brain tissue for use with 10x Genomics Single Cell protocols. The surgical dissection of embryonic mouse tissue is not described here.

Additional Guidance

Consult Demonstrated Protocol Cell Preparation Guide (Document CG00053) for Tips & Best Practices on handling cells and Technical Note Guidelines for Accurate Target Cell Counts using 10x Genomics Single Cell Solutions (Document CG000091) for determining accurate cell counts.

Cells carry potentially hazardous pathogens. Follow tissue and material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage, and disposal of biological materials.

Cell Sourcing

This protocol was demonstrated using E18 Mouse Combined Cortex, Hippocampus, and Ventricular Zone neural tissues from BrainBits (PN-C57EHCV).

Preparation – Buffers

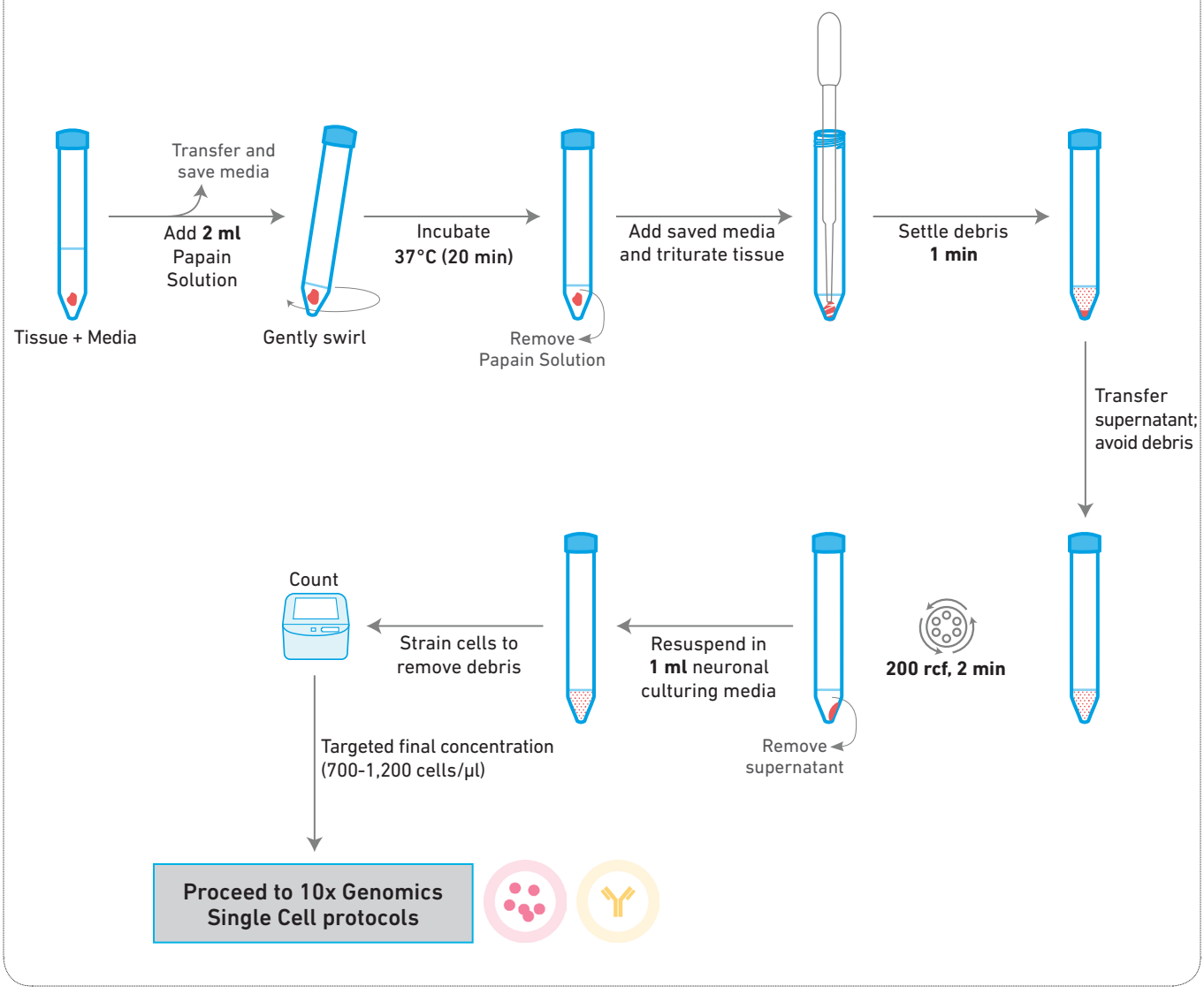
Solutions	Composition
Papain Solution	2 mg/ml Papain in Dissociation Solution (HECA5) • Add HECA5 to the Papain vial for a 2 mg/ml final concentration. • Transfer the mix to a 15-ml tube and incubate at 37°C (in water bath or equivalent) for 10 min prior to use.
Additional Reagents	
NbActiv1 – Neuronal Culture Medium (maintain at 37°C)	

Specific Reagents & Consumables

Vendor	Item	Part Number
Thermo Fisher Scientific	Trypan Blue Stain (0.4%)	T10282
	Live/DEAD Viability/Cytotoxicity Kit for mammalian cells	L3224
	Countess II FL Automated Cell Counter	AMAQAF1000
	Countess II FL Automated Cell Counting Chamber Slides	C10228
Miltenyi	MACS SmartStrainers, 30 µm	H13680-0040
VWR	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 15 ml	21008-103
BrainBits	E18 Mouse Combined Cortex, Hippocampus & Ventricular Zone	C57EHCV
	NbActiv1 Neuronal culturing medium	NbActive1 100
	Papain/HE (Papain plus 5 ml HE-CA) Dissociation Solution	PAP/HE
	Sterile 9" Silanized Glass Pasteur Pipette	FPP

Protocol Overview

Mouse Neural Tissue Dissociation 30-45 min



Protocol

Tissues and materials from BrainBits were stored according to manufacturer's recommendations prior to starting the protocol.

a. Transfer the tissue along with the Hibernate E/B27/ GlutaMAX (HEB) media to a 15-ml centrifuge tube.

b. Transfer and save the HEB media from the tissue to a new 15-ml tube, leaving only enough medium to cover the tissue. Keep the medium for step e.

c. Add **2 ml** Papain Solution to the tissue and incubate at **37°C** for **20 min**. Gently swirl to mix, repeat 3x during the incubation.

d. Remove the Papain Solution without disrupting the tissue at the bottom, leaving only enough solution to cover the tissue.

e. Add the medium saved at step b to the tissue.

f. **Triturate the tissue:** Aspirate the tissue with the medium into a fire polished silanized Pasteur pipette and **immediately** dispense the contents back into the tube. Repeat for a maximum of 10x.

g. Allow the tissue debris to settle for **1 min**.

h. Transfer the supernatant containing cells to a new 15-ml centrifuge tube, leaving tissue debris at the bottom.

i. Centrifuge cells at **200 rcf** for **2 min**.

j. Remove the supernatant without disturbing the cell pellet. Leave only enough media to cover the cell pellet.

k. Using a regular-bore pipette tip, resuspend the cell pellet in **1 ml** pre-warmed neuronal culturing medium (NbActiv1) by gently pipetting 20x or until cells are completely suspended.

l. Pass the cell suspension through a 30 µM MACS SmartStrainer. Determine the concentration using a Countess II Automated Cell Counter or a hemocytometer. The targeted final cell concentration is 700-1,200 cells/µl.

m. If necessary, dilute the cell suspension with additional neuronal culturing medium until the target cell concentration is reached.

n. Once the final cell concentration is achieved, place cells on ice.

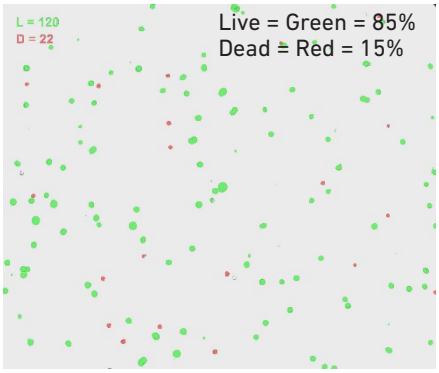
o. Proceed **immediately** with the 10x Genomics Single Cell protocols.

Results

The percent viability of mouse embryonic brain cells obtained by following this protocol ranges from 85-92% as assessed by trypan blue staining and/or live/dead viability staining.

Trypan Blue Staining on Countess II

Live/Dead Viability Staining



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