

## 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) <ul style="list-style-type: none"> <li>Add HECA5 to the Papain vial for a 2 mg/ml final concentration.</li> <li>Transfer the mix to a 15-ml tube and incubate at 37°C (in water bath or equivalent) for 10 min prior to use.</li> </ul>

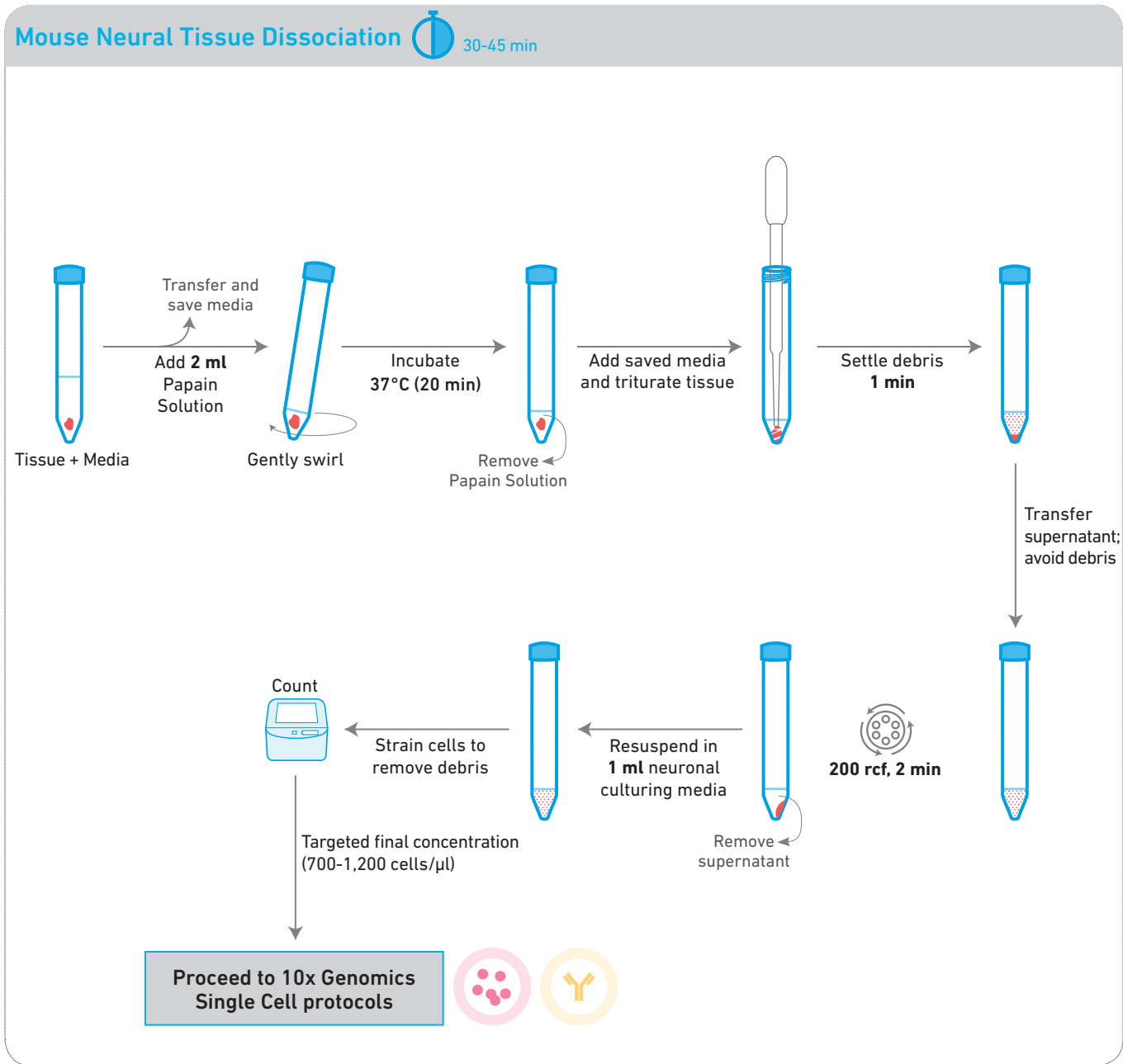
### Additional Reagents

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



## 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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