10x Genomics®

Sample Preparation Demonstrated Protocol

Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing

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Manual Part Number

CG00055 Rev A

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Demonstrated Protocol

Dissociation of Mouse Embryonic Neural Tissue for Single Cell RNA Sequencing

1. Overview

This Demonstrated Protocol outlines how to obtain a single cell suspension from embryonic mouse brain tissue in preparation for use in 10x Genomics® Single Cell Protocols. The surgical dissection of embryonic mouse tissue is not described here.

2. Getting Started

2.1. Tips & Safety

Best practices for handling any cell line include using sterile technique, nuclease-free reagents and consumables, minimizing pipetting steps, and using wide-bore pipette tips where possible to minimize cell damage.

To determine accurate cell counts, best practices include sampling the cell suspension at least twice and carrying out at least two counts on each sample (i.e. a minimum of four counts in total, based on two independent draws from the cell suspension).

CRITICAL! *Follow tissue and material supplier recommendations and local laboratory procedures and regulations for the safe handling, storage and disposal of biological materials.*

2.2. Materials

Supplier	Description	Part Number (US)
-	Refrigerated Benchtop Centrifuge for 15 ml and 50 ml tubes	-
	Microcentrifuge for 2 ml LoBind tubes	-
	Heated Water Bath, 2l	-
Miltenyi	MACS SmartStrainers, 30 µm	130-098-458
Thermo Fisher Sci	Trypan Blue Stain (0.4%) for use with the Countess® Automated Cell Counter	T10282
	Countess® II Automated Cell Counter	AMQAX1000
	Countess® II Automated Cell Counting Chamber Slides	C10228
	Live/DEAD Viability/Cytotoxicity Kit for mammalian cells	L3224
Eppendorf	DNA LoBind Tubes 2.0 ml*	022431048
VWR	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 50 ml	82018-050
	Sterile Polypropylene Centrifuge Tubes with Flat Caps, 15 ml	21008-103

*No substitutions are allowed. Items have been validated by 10x Genomics and are required for Single Cell workflow, training and system operations.

2.3. Specific Tissue Sourcing & Materials

NOTE

The Protocol was developed using tissue and materials from BrainBits (www.brainbitsllc.com). Materials were stored according to manufaturer's recommendations prior to starting the Protocol. Fresh mouse brain tissue was shipped on cold packs and used immediately upon receipt.

Tissue / Material	Description	BrainBits SKU
Neuronal Tissue	E18 Mouse Combined Cortex, Hippocampus and Ventricular Zone	msHPCTX
Neuronal Culturing Medium	For Primary Neuron Cultures	NbActiv1
Dissociation Solution	Hibernate E Minus Calcium (5 ml)	HE-Ca5
Papain	-	PAP
Fire Polished Silanized Pasteur Pipette	-	FPP

2.4. Preparation – Buffers & Media

a) Warm a water bath to 37°C.

b) Pre-warm neuronal culturing medium (NbActiv1) to 37°C (in water bath or equivalent).

c) Prepare papain solution for tissue dissociation by dissolving papain to a final concentration of **2 mg/ml** in dissociation solution (HE-Ca). Add **3.2 ml** HE-Ca to a vial of **6.4 mg** papain and mix. Transfer the mix to a 15 ml conical tube and incubate at **37°C** for **10 min** prior to use.

3. Dissociation of Mouse Embryonic Neural Tissue

3.1. Dissociation Protocol

a) Using a 1000 µl **wide-bore** pipette tip, gently transfer the tissue along with the Hibernate E®/B27®/GlutaMAX™ (HEB) medium to a new 15 ml conical tube and wait until the tissue is settled at the bottom of the tube.

b) Transfer the HEB medium from the tissue to a new 15 ml conical tube, leaving only enough medium to cover the tissue. Keep the HEB 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 2 – 3 times during the incubation.

d) Remove and discard the papain solution without disrupting the tissue at the bottom, leaving only enough solution to cover the tissue.

e) Add the HEB medium saved from step b back to the tissue.

f) Aspirate the tissue with the HEB medium into a fire polished silanized Pasteur pipette and immediately dispense the contents back into the tube. Triturate with 10 – 15

passes of the tissue through the pipette. The goal is to achieve ~85% tissue dissociation.

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

h) Transfer the supernatant containing dispersed cells to a new 15 ml conical tube, leaving tissue debris at the bottom.

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

j) Remove and discard 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 20 times or until cells are completely suspended. This volume results in a cell concentration of ≥2000 cells/µl.

l) Place a 30 µm cell strainer on top of a 5 ml tube and filter the resuspended cells into the 5 ml tube.

m) Determine the cell concentration using a Countess® II Automated Cell Counter. The target concentration is 2000 cells/µl (2 x 10^6 cells/ml).

n) If necessary, dilute the cells with additional neuronal culturing medium until the target cell concentration is reached.

o) Once the target cell concentration is obtained, place the cells on ice.

p) Proceed with the 10x Genomics® Single Cell Protocol.

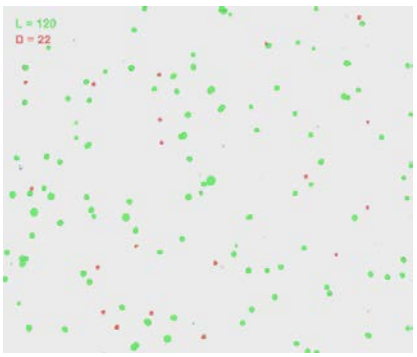
4. Typical Viability Results

The typical percent viability of mouse embryonic brain cells obtained by following this Protocol ranges from mid 80s to low 90s (85 – 92%) based on both trypan blue staining and live/dead staining (using a Thermo Fisher Live/Dead® Viability/Cytotoxicity Kit, for example).

Trypan Blue Staining

Live = Green = 89%

Dead = Red = 11%



Live/Dead Viability Staining

Live = Green = 85%

Dead = Red = 15%