Jan 11, 2026

Protocol for Mouse Brain and SH-SY5Y Cell Sample Preparation for Electron Microscopy

DOI
https://dx.doi.org/10.17504/protocols.io.n92ld6377g5b/v1

Ali Ghoochani[1,2,3,4], Monther Abu-Remaileh[1,2,3,4]

[1]Department of Chemical Engineering, Stanford University, Stanford, CA 94305, USA;
[2]Department of Genetics, Stanford University, Stanford, CA 94305, USA;
[3]The Institute for Chemistry, Engineering and Medicine for Human Health (Sarafan ChEM-H), Stanford University, Stanford, CA 94305, USA;
[4]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD, 20815, USA

asap

Ali Ghoochani
Stanford University

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld6377g5b/v1

Protocol Citation: Ali Ghoochani, Monther Abu-Remaileh 2026. Protocol for Mouse Brain and SH-SY5Y Cell Sample Preparation for Electron Microscopy . **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld6377g5b/v1

Protocol status: Working
We use this protocol and it's working

Created: July 09, 2025

Last Modified: January 12, 2026

Protocol Integer ID: 222035

Keywords: sy5y cell sample preparation for electron microscopy, sy5y cells for electron microscopy, sy5y cell sample preparation, sy5y cell, electron microscopy, protocol for mouse brain, preparation of mouse, microscopy, mouse brain, cortical tissue, cell, mouse, tissue, brain

Abstract

This protocol outlines the preparation of mouse cortical tissue and SH-SY5Y cells for electron microscopy (EM).

Materials

- 4% Paraformaldehyde (PFA)
- 2% Glutaraldehyde
- 5% and 30% Sucrose solutions
- O.C.T. compound (TissueTek)
- Cryostat or vibratome
- Falcon tubes (15 mL)
- Aclar coverslips
- 1× DPBS
- Serum-depleted DMEM
- BSA-20 nm gold conjugate

Procedure

1 **Mouse Brain Tissue Preparation:**

1.1 Immediately after euthanasia, harvest mouse brains.

1.2 Fix cortices overnight at 4°C in 4% PFA and 2% glutaraldehyde.

1.3 Transfer tissues to a solution of 4% PFA, 2% glutaraldehyde, and 5% sucrose. Incubate overnight at 4°C.

1.4 Place tissues in 30% sucrose solution in 15 mL Falcon tubes at 4°C until they sink.

1.5 Embed sunk tissues in O.C.T. compound, freeze, and store at –80°C.

1.6 Cut coronal sections at a thickness specified by the electron microscopy facility using a cryostat or vibratome.

Note

This step depends on the specific requirements of the electron microscopy facility. Ensure that the appropriate fixative and section thickness are used according to the facility's guidelines.

Procedure

2 **SH-SY5Y Cell Preparation for EM:**

2.1 Seed 0.2 × 10^6 SH-SY5Y wild-type, SLC45A1-KO, and rescue cells onto Aclar coverslips.

2.2 Wash cells with 1× DPBS and incubate in serum-depleted DMEM for 72 h.

2.3 Treat cells with 0.2 g/100 mL BSA-20 nm gold conjugate to label lysosomes, 9 hours before completing the 72-hour incubation period.

2.4 Wash cells with DPBS and fix samples with w/2% glutaraldehyde & 4% paraformaldehyde in 0.1M Sodium Cacodylate buffer, pH 7.4. Then move samples to 4°C. Ship samples to EM facility for further process.

Note

This step depends on the specific requirements of the electron microscopy facility. Ensure that the appropriate fixative according to the facility's guidelines