Feb 16, 2026

Acute Midbrain Slice Preparation

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DOI
https://dx.doi.org/10.17504/protocols.io.q26g776dkgwz/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.q26g776dkgwz/v1

Protocol Citation: Nicola Berretta, Ezia Guatteo, Nicola Biagio Mercuri 2026. Acute Midbrain Slice Preparation . **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g776dkgwz/v1

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

Created: February 16, 2026

Last Modified: February 16, 2026

Protocol Integer ID: 243379

Keywords: acute midbrain slice preparation, µm horizontal midbrain slice, using chilled nmdg, chilled nmdg, protective cutting solution, graded sodium reintroduction, based protective cutting solution, sodium reintroduction, preparation, electrophysiological recording, storage in hepe

Abstract

This protocol describes preparation of 250 µm horizontal midbrain slices containing the VTA using chilled NMDG-based protective cutting solution, followed by graded sodium reintroduction and storage in HEPES-based aCSF prior to electrophysiological recording.

Materials

NMDG-Based Cutting Solution (mM)
92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 0.5 CaCl2, 10 MgSO4. Equilibrate with 95% O2 - 5% CO2, pH 7.3. Keep chilled.

HEPES-Based Storage Solution (mM)
92 NaCl, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Na-pyruvate, 2 CaCl2, 2 MgSO4. Equilibrate with 95% O2 - 5% CO2, pH 7.3. Store at room temperature.

Step-by-Step Procedure

1 Anesthetize the mouse with halothane and decapitate rapidly.

2 Remove the brain and immediately place it into chilled carbogenated NMDG solution.

3 Mount the brain for horizontal slicing.

4 Cut 250 µm horizontal slices containing the midbrain using a vibratome.

5 Transfer slices to NMDG solution at 33.0 C and incubate for 15 min.

6 Gradually reintroduce sodium by adding increasing volumes of 2 M NaCl solution every 5 min for 40 min total.

7 Transfer slices to HEPES-based aCSF at room temperature.

8 Allow at least 1 h recovery before recording.