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Preparation of Holding ACSF (artificial cerebrospinal fluid) for slice electrophysiology

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Protocol status: Working

We use this protocol and it's working

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Abstract

Hnasko lab- preparation of Holding ACSF

Holding ACSF is used for incubating brain slices prior to cell-attached or whole-cell electrophysiological recordings of neurons.

Materials

NaCl (Fisher Scientific, cat# BP358)

KCl (Fisher Scientific, cat# BP366)

NaH₂PO₄ (Fisher Scientific, cat# BP329)

D-glucose (Sigma Aldrich, cat# G8270)

CaCl₂ (Fisher Scientific, cat# BP510)

MgSO₄ (Fisher Scientific, cat# M80)

NaHCO₃ (Fisher Scientific, cat# BP328)

Thiourea (Sigma Aldrich, cat# T8656)

Na-Ascorbate (Sigma Aldrich, cat# A7631)

Na-Pyruvate (Sigma Aldrich, cat# P5280)



- 1 Prepare 10X stock solution.
 - 1.1 Composition of  1 L of 10X Holding ACSF (in mM): 1150 NaCl, 25 KCl, 12.3 NaH₂PO₄, 100 d-glucose, 20 CaCl₂ and 20 MgSO₄.
 - 1.2 Composition of  1 L of 10X Holding ACSF (in g): 67.2 NaCl, 1.86 KCl, 1.47 NaH₂PO₄, 18.02 d-glucose, 2.94 CaCl₂ and 4.93 MgSO₄.
- 2 Dissolve all salts in  1 L of ddH₂O to make 10X stock solution of Holding ACSF.
- 3 Dilute 10X solution to 1X for use in experiments.
 - 3.1 Dilute  100 mL of 10X solution to ~  800 mL with ddH₂O and add (in mM): 26 NaHCO₃, 2 thiourea, 5 Na-ascorbate and 3 Na-pyruvate; (in g): 2.18 NaHCO₃, 0.15 thiourea, 0.98 Na-ascorbate and 0.33 Na-pyruvate.
 - 3.2 Add ddH₂O to final volume of  1 L .
- 4 Bubble the solution with carbogen (95% O₂ 5% CO₂) for ~10 minutes.
- 5 After bubbling, measure and adjust pH of solution to ~  7.3 with 12 N HCl (<1 ml).
- 6 Refrigerate until use.