Sep 15, 2022

Protocol for ex vivo patch clamping

Maia Datunashvili[1]

[1]Northwestern university

dx.doi.org/10.17504/protocols.io.261ge361ol47/v1

divya.darwinarulseeli

ABSTRACT

Protocol for ex vivo patch clamping

DOI

dx.doi.org/10.17504/protocols.io.261ge361ol47/v1

PROTOCOL CITATION

Maia Datunashvili 2022. Protocol for ex vivo patch clamping. **protocols.io** https://protocols.io/view/protocol-for-ex-vivo-patch-clamping-cgp2tvqe

CREATED

Sep 15, 2022

LAST MODIFIED

Sep 15, 2022

PROTOCOL INTEGER ID

70106

MATERIALS TEXT

Reagents, instruments and materials

- Ketamine / xylazine cocktail
- Fine science surgical tools, including scalpel, scissors, forceps
- Petri dishes
- Vibratome
- Blade

Solutions:

- Sucrose-based artificial cerebrospinal fluid (sucrose aCSF in mM): 230 sucrose, 26 $NaHCO_3$, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 10 $MgSO_4$, 10 glucose, 1 Na-pyruvate, and 0.005 L-glutathione.
- Standard aCSF (in mM): 26 $NaHCO_3$, 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 2 $MgSO_4$, 10 glucose, 1 Na-pyruvate, and 0.005 L-glutathione.
- Synthetic interstitial fluid (SIF) warmed to 35C, equilibrated with 95% O_2 and 5% CO_2 and containing (in mM): 26 $NaHCO_3$, 126 NaCl, 3 KCl, 1.25 NaH_2PO_4, 1.6 $CaCl_2$, 1.5 $MgSO_4$, and 10 glucose.

Equipment Setup

- Vibratome

Preparation of acute brain slices

1 Chill sucrose aCSF up to 4 degrees and then keep it on the ice and bubble continuously by carbogen.

2 Place 150 ml standard aCSF in holding chamber. Warm it up to 34oC in water bath and bubble continuously by carbogen.

3 After 3-5 weeks of the viral injection, anesthetize the animal briefly with Isoflurane and then make IP injection of ketamine/xylazine cocktail (87/13 mg/kg i.p.).

4 Perfuse the animal transcardially with 4-6 ml ice-cold sucrose aCSF.

5 Open the scalp, block the brain between the hemispheres and transfer it to the petri dish filled with ice-cold oxygenated sucrose solution.

6 Glue the brain on the specimen plate and cut 250 um thickness slices on the vibratome.

Citation: Maia Datunashvili Protocol for ex vivo patch clamping https://dx.doi.org/10.17504/protocols.io.261ge361ol47/v1

7 Wash the slices with standard aCSF and transfer them to the holding chamber. Keep the slices 30 min at 340C and then at room temperature.

Citation: Maia Datunashvili Protocol for ex vivo patch clamping https://dx.doi.org/10.17504/protocols.io.261ge361ol47/v1