

### Sodium Current Measurements in HEK293 Cells

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**[Abstract]** This protocol is used to measure sodium currents from heterologously transfected cells lines, such as HEK293 cells. Standard whole cell patch clamp technique is used to assess ion channel function. This protocol has been used to study cardiac type Na<sub>v</sub>1.5 sodium channel, and in particular to compare wild-type and mutant channels related to Brugada Syndrome (BrS). Mutations related to BrS provoke a loss of function of the cardiac sodium channel. This is evidenced by a reduction of sodium current density and/or alterations of the activation or inactivation kinetic parameters, such as a slow recovery from inactivation. These effects could explain the alteration of the cardiac action potential that leads to the characteristic ST elevation observed in the electrocardiogram of the Brugada Syndrome patients. This protocol, with some variations, is suitable for studying other cardiac arrhythmias related to alterations in the cardiac type sodium channel function as well as for studying other voltage-dependent sodium channels.

#### **Materials and Reagents**

1. Human embryonic kidney HEK293 cells (Health Protection Agency Culture Collections, catalog number: 96121229)
2. Vector/s: mammalian expression vectors harboring the cDNA of interest (*i.e.* pcDNA3 + SCN5A)
3. Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, catalog number: D6546)
4. Fetal Bovine Serum (Sigma-Aldrich, catalog number: F4135)
5. Penicillin-streptavidin (Life Technologies, Gibco<sup>®</sup>, catalog number: 15140-122)
6. GlutaMAX<sup>™</sup> (Life Technologies, Gibco<sup>®</sup>, catalog number: 35050-038)

7. 0.05% Trypsin-EDTA (Life Technologies, Gibco<sup>®</sup>, catalog number: 25300-062)
8. Dulbecco's Phosphate Buffered Saline (Sigma-Aldrich, catalog number: D8662)
9. GeneCellin<sup>™</sup> Transfection Reagent (BioCellChallenge, catalog number: GC-1000)
10. Opti-MEM<sup>®</sup> Reduced Serum Media + GlutaMAX<sup>™</sup> (Life Technologies, Gibco<sup>®</sup>, catalog number: 31985-062)
11. Sticky wax in bars (Cera de Reus, catalog number: 25005001)
12. Sodium chloride (NaCl) (Sigma-Aldrich)
13. Potassium chloride (KCl) (Sigma-Aldrich)
14. Cesium chloride (CsCl) (Sigma-Aldrich)
15. Calcium chloride (CaCl<sub>2</sub>) (Sigma-Aldrich)
16. Magnesium chloride (MgCl<sub>2</sub>) (Sigma-Aldrich)
17. N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (Sigma-Aldrich)
18. Ethylene glycol-bis (2-amino-ethylether)-N,N,N',N'-tetra-acetic acid (EGTA) (Sigma-Aldrich)
19. Adenosine 5'-triphosphate magnesium salt (ATP-Mg<sup>2+</sup>) (Sigma-Aldrich)
20. Sodium hydroxide (NaOH) (Sigma-Aldrich)
21. Cesium hydroxide (CsOH) (Sigma-Aldrich)
22. Glucose (Sigma-Aldrich)
23. Bath solution (see Recipes)
24. Pipette solution (see Recipes)

### **Equipment**

1. Inverted microscope Nikon Eclipse Ti fitted for epifluorescence (Nikon Instruments Inc.)
2. Glass capillary PC-10 Puller (Narishige International USA Inc.)
3. Micromanipulator MP-285 (Sutter Instrument Co.)
4. Vapor pressure Osmometer VAPOR 5520 (Wescor Inc.)
5. Axopatch 200B Capacitor Feedback Patch Clamp Amplifier (Molecular Devices)
6. Axon Digidata 1440A Data Acquisition System (Molecular Devices)
7. CO<sub>2</sub> incubator
8. Nunclon<sup>®</sup> cell culture dishes 35 x 10 mm (Sigma-Aldrich, catalog number: D7804)
9. Glass capillaries (Brand GmbH + CO KG, catalog number: 7493-21)

### **Software**

1. pCLAMP 10.2 Electrophysiology Data Acquisition and Analysis Software (Molecular Devices)

2. OriginPro 8 software (OriginLab Corporation)

## **Procedure**

### A. Cell culture and transfection

1. The day before transfection, plate HEK293 cells in 35 mm dishes at a density that will result in 70% confluence 24 h later (this is  $2.1 \times 10^5$  cells but it may vary depending on how fast your cell line grows), and maintain in a CO<sub>2</sub> incubator at 37 °C in DMEM supplemented with 10% Fetal Bovine Serum (FBS), 1% Penicillin-streptomycin and 1% GlutaMAX™.

2. Perform the transfection for each 35 mm dish of HEK293 cells with a mix of 200 µl Opti-MEM® Medium, 3 µg (in total) of the vectors of interest and 4 µl of GeneCellin™ Transfection Reagent. The inclusion of a vector harboring the cDNA encoding a fluorescent protein will allow the identification of transfected cells.

*Note: Other transfection reagents and methods have been successfully used for transfecting sodium channels.*

3. Twenty-four hours after transfection, wash the each dish with 1.5 ml DPBS, then add 0.2 ml 0.05% Trypsin-EDTA and incubate at 37 °C for 2 min, cells should look separated from each other, add 1.5 ml of DMEM to the dish, disperse with a fine tip pipette and plate 200-300 µl in a new dish with 1.5 ml of media.

*Note: You should get individual cells for recording.*

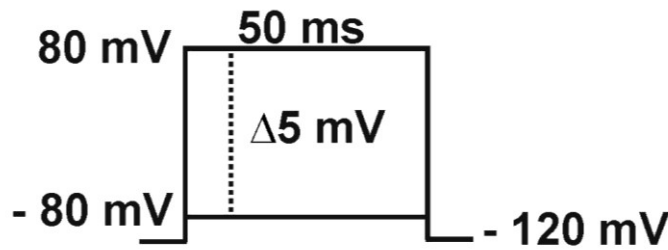
### B. Patch-clamp procedure (48 h after transfection)

1. Pull pipettes from glass capillaries and finely coat the tip with melted dental wax (be careful not to occlude the tip hole). Their resistance should range from 2 to 4 MΩ when filled with the Pipette solution.
2. Remove DMEM from the transfected cells dish that you will use and, after rinsing the cells twice with the Bath solution (approximately 3 ml in total), fill it to about one third of the height with the same solution.
3. Choose the cells that you will record from individual fluorescent cells.
4. Fill the pipette with the pipette solution and place it in the electrode holder. Lower the pipette to place it in the Bath solution. After compensating offsets, approach the pipette to the chosen cell with the help of the remote micromanipulator to form a high resistance cell-attached seal.
5. Once the seal is formed and the whole cell configuration is established, compensate series resistance at 80-90%.

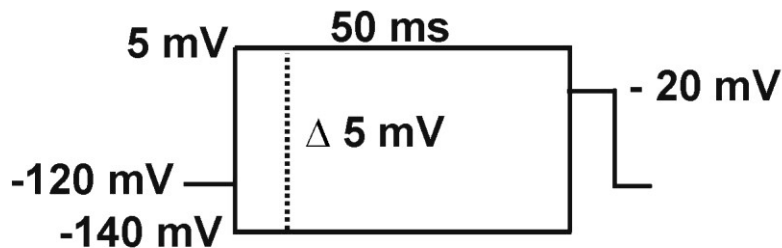
6. Wait five minutes before starting to record. This allows the cell content to equilibrate with the pipette solution.
7. For acquisition, set your filter at 5 kHz and your sampling rate at 20-25 kHz.

C. Sodium measurements protocols

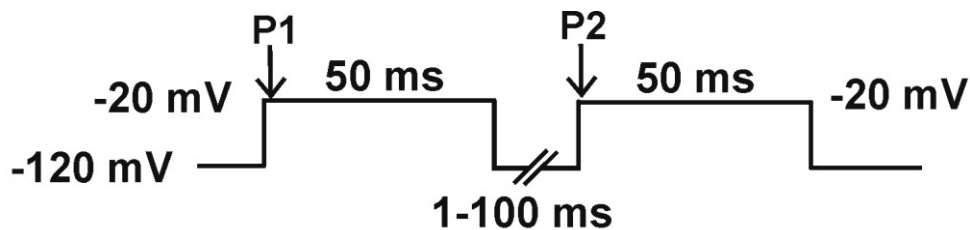
1. Macroscopic sodium current: Currents are elicited by 50 ms depolarizing steps (from -80 to +80 mV in 5 mV increments) from a holding potential of -120 mV.



2. Steady-state inactivation ( $h_{\infty}$ ): Current is measured with -20 mV pulses (20 ms), following 50 ms pre-pulses to different potentials (-140 to +5 mV in 5 mV increments).



3. Recovery from inactivation: current is elicited by a -20 mV, 20 ms, pulse (P2), preceded by a 50 ms depolarizing pre-pulse to -20 mV (P1) from a holding potential of -120 mV, followed by a hyperpolarizing pulse to -120 mV of increasing duration (1-100 ms).





interval times. The recovery from inactivation curve is fitted to a mono-exponential function to obtain the time constants ( $t$ ).

6. Slow inactivation: Peak current is measured at P2 and P1. The P2/P1 ratio is plotted against the depolarizing pulse interval times. The slow inactivation curve is fitted to a mono-exponential function to obtain the slow inactivation constant ( $t$ ).

## **Recipes**

1. Bath solution

140 mM NaCl

3 mM KCl

10 mM HEPES

1.8 mM CaCl<sub>2</sub>

1.2 mM MgCl<sub>2</sub>

The pH is adjusted to 7.4 with NaOH

Osmolality is adjusted by the addition of glucose to approximately 325 mOsm

2. Pipette solution

130 mM CsCl

1 mM EGTA

10 mM HEPES

10 mM NaCl

2 mM ATP-Mg<sup>2+</sup>

The pH is adjusted to 7.2 with CsOH

Osmolality is adjusted by the addition of glucose to approximately 308 mOsm.

*Note: The difference in osmolality between Pipette and Bath solutions should be near 5%.*

## **Acknowledgments**

This protocol is adapted from Riuro *et al.* (2013) and Tarradas *et al.* (2013).

## **References**

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2. Tarradas, A., Selga, E., Beltran-Alvarez, P., Perez-Serra, A., Riuro, H., Pico, F., Iglesias, A., Campuzano, O., Castro-Urda, V., Fernandez-Lozano, I., Perez, G. J., Scornik, F. S. and Brugada, R. (2013). [A novel missense mutation, I890T, in the pore region of cardiac sodium channel causes Brugada syndrome.](#) *PLoS One* 8(1): e53220.

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1. Sakmann, Bert and Neher, Erwin. (1995). Single-Channel Recording. Springer-Verlag, 2<sup>nd</sup> Edition. New York.
2. Molleman, Areles (reprinted 2008). Patch Clamping. An Introductory guide to patch clamp electrophysiology. John Wiley and Sons Ltd. England: West Sussex.