



Jul 11, 2019

SPARC Retrograde Neuroanatomical Tracing of Phrenic Motor Neurons Using Intrapleural Injections of Cholera Toxin B Fragment

 In 1 collection

DOI

dx.doi.org/10.17504/protocols.io.2kpgcvn

Latoya Allen¹, Marissa Ciesla¹, Yasin Seven¹, Elisa Gonzalez-Rothi¹, Gordon Pool¹

¹University of Florida

SPARC

Tech. support email: info@neuinfo.org



Elisa Gonzalez-Rothi

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.2kpgcvn>

Protocol Citation: Latoya Allen, Marissa Ciesla, Yasin Seven, Elisa Gonzalez-Rothi, Gordon Pool 2019. SPARC Retrograde Neuroanatomical Tracing of Phrenic Motor Neurons Using Intrapleural Injections of Cholera Toxin B Fragment. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.2kpgcvn>



License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: May 03, 2019

Last Modified: July 11, 2019

Protocol Integer ID: 22895

Keywords: Cholera toxin B fragment, CT-B, phrenic, intrapleural, retrograde, neuroanatomical tracing, labeling bilateral phrenic motor neuron pool, phrenic motor neuron, cervical spinal cord, using intrapleural injection

Abstract

This protocol describes the procedure for retrogradely labeling bilateral phrenic motor neuron pools in the cervical spinal cord using intrapleural injections of cholera toxin B fragment

Troubleshooting

- 1 Anesthesia is induced in a closed chamber (3.5%isoflurane in 100%O₂) then transfered to a heated surgical station and anesthesia is maintained via nose cone at 2-2.5%
- 2 Sterilized 25 μ L Hamilton syringes with 9.52-mm sterile needles are prepared to administer CtB intrapleurally in rats.
- 3 Cholera toxin B fragment (CtB) is prepared as follows: 0.2% weight/volume; dissolved in sterile H₂O; Calbiochem, Billerica, MA.
- 4 25 μ L of CtB is loaded into a 25- μ L Hamilton syringe and 12.5 μ L is injected per side (25 μ L total per animal).
- 5 Prep injection site for injection using aseptic methods:
 - 5.1 shave and scrub injection sites per Institutionally approved animal protocol
- 6 Rats were turned sideways to inject CtB at the 5th intercostal space at a depth of ~6 mm.
- 7 Following injections, discontinue isoflurane and allow rat to recover fully while monitoring for signs of respiratory compromise or distress