JUL 12, 2019

OPEN ACCESS

DOI:
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>

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

Created: May 03, 2019

Last Modified: Jul 12, 2019

PROTOCOL integer ID:
22895

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

In 1 collection

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

¹University of Florida

SPARC

Tech. support email: info@neuinfo.org

Elisa Gonzalez-Rothi

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

Keywords: Cholera toxin B fragment, CT-B, phrenic, intrapleural, retrograde, neuroanatomical tracing

- 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