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Staining and imaging of mouse submandibular ganglion by α -bungarotoxin and nanosensor

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Protocol status: Working

We use this protocol and it's working

Created: January 09, 2019

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Keywords: acetylcholine receptors in isolated submandibular ganglion, acetylcholine receptor, isolated submandibular ganglion, submandibular ganglion, bungarotoxin, nanosensor, nanosensor this protocol, imaging of mouse

Abstract

This protocol describes how to label acetylcholine receptors in isolated submandibular ganglion from mice using α -bungarotoxin and nanosensor formulated by the Clark lab.

Materials

MATERIALS

⊗ 100% formalin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F1635-500ML**

⊗ α -Bungarotoxin Alexa Fluor 647 conjugated **Thermo Fisher Scientific Catalog #B35450**

⊗ PBS pH 7.4 **Thermo Fisher Scientific Catalog #10010023**

ChAT^{BAC}-eGFP transgenic mice from The Jackson Laboratory.

Stock number: 007902

Protocol materials

⊗ 100% formalin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F1635-500ML**

⊗ α -Bungarotoxin Alexa Fluor 647 conjugated **Thermo Fisher Scientific Catalog #B35450**

⊗ PBS pH 7.4 **Thermo Fisher Scientific Catalog #10010023**

Extraction of submandibular ganglion

1. Enthanize animals using CO₂.
2. Make a midline incision in the neck and expose the salivary glands.
3. Separate the pair of salivary glands and expose the salivary ducts.
4. Remove connective tissues surrounding salivary ducts and cut the ducts from where they enter salivary glands to where they enter digastric muscles. The submandibular ganglion are attached to cutted ducts.

Expected result

Well isolated salivary ducts with at least one submandibular ganglion attached

Staining of submandibular ganglion by α -bungarotoxin and nanosensor (formulated by Clark Lab)

- 2
 1. Rinse submandibular ganglion with PBS for  00:20:00
 2. Soak submandibular ganglion in  500 μ L  0.000002 Mass Percent
 α -Bungarotoxin Alexa Fluor 647 conjugated **Thermo Fisher Scientific Catalog #B35450**
for  00:30:00 .
Alternatively, soak submandibular ganglion in  500 μ L nanosensor solution formulated by Clark Lab for  00:30:00
 3. Rinse submandibular ganglion with PBS for  00:20:00 .

Imaging of submandibular ganglion

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 1. Place the submandibular ganglion on a glass slide and cover it with cover slip.
 2. Image the whole tissue using a Zeiss LSM 700 confocal. Use 488 nm laser line for GFP and Oregon Green 488 channel, 555 nm laser line for pHAb, and 639 nm laser line for bungarotoxin-Alexa Fluor 647 channel. Set laser power at 2% or at the appropriate power intensity that cellular structures can be clearly seen.



Software

ZEN pro

NAME

ZEISS

DEVELOPER

<https://www.zeiss.com/microscopy/us/products/microscope-software/zen.html>

SOURCE
LINK