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

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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.

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MATERIALS TEXT

MATERIALS

100% formalin

Sigma Catalog #F1635-500ML

α-Bungarotoxin Alexa Fluor 647 conjugated **Thermo Fisher**

Scientific Catalog #B35450 Step 2

PBS pH 7.4 **Thermo Fisher**

Scientific Catalog #10010023

ChAT^{BAC}-eGFP transgenic mice from The Jackson Laboratory.
Stock number: 007902

Extraction of submandibular ganglion

1 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.

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 µl** **[M]0.000002 Molarity (M)**

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

for **00:30:00** .

Alternatively, soak submandibular ganglion in **500 µl** nanosensor solution formulated by Clark Lab for **00:30:00**

3. Rinse submandibular ganglion with PBS for **00:20:00** .

Imaging of submandibular ganglion

3 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 Oregan 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.

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