Standard Operating Procedure (SOP)

Aim of SOP (tick box)

☐ Munition detection or identification	☐ Toxicity
☐ Sampling	☐ In situ exposure studies
☒ Sample preparation	☐ Bioassays
☐ Chemical analysis	
☐ Bioindicators/biomarkers	

9. Homogenization of fish muscle and mussel gill tissues

version 1.1

Aino Ahvo

Finnish Environment Institute (SYKE)
Latokartanonkaari 11, 00790 Helsinki, Finland
email: aino.ahvo@ymparisto.fi

Scope

This SOP describes the method of preparing samples for acetylcholinesterase (AChE) (see AChE SOP) analysis in biological samples. The method is based on references 1 and 2 and has been modified for mussel and fish samples by SYKE. It has been used the EU-funded project Decision Aid for Marine Munitions (DAIMON).

Summary of the method/SOP

Tissue and cells are broken mechanically in a buffer solution, the sample is centrifuged and the resulting supernatant is used in the assays.

Safety aspects

Normal laboratory safety rules should be applied.

Documentation

All samples should have a unique code and label. Pipetting errors or other anomalies that could affect the interpretation of the results should be recorded.

Methods

Matrix: Fish muscle and mussel gill tissue.

Equipment: Weighing balance (1 mg accuracy); homogenizer (e.g., Qiagen TissueLyser); Eppendorf-tube centrifuge with cooling possibility and 10 000 x g; basic laboratory equipment (2 ml eppendorf tubes, decanters).

Table 1: Solutions used in the analysis.

solution	

sodium phosphate buffer	20 mM Na_2HPO_4 + 20mM NaH_2PO_4 + 0,1 % (v/v) Triton-X100 in water, pH 7.0

Table 2: Reagents needed in the procedure.

reagent	**CAS-number**
Na_2HPO_4	7558-80-7
NaH_2PO_4	7558-79-4
Triton-X100	9002-93-1

Procedure: Tissues are homogenized in individual 2 ml eppendorf tubes in sodium phosphate buffer. The volume of the buffer (in µl) is calculated as six times the weight (for fish muscle) and two times the weight (for mussel gills) of the tissue sample in mg. Samples are homogenized in 30 Hz frequency for 2 x 45 sec. After homogenization the samples are centrifuged for 20 min in 10,000 g at 4°C and aliquots of the supernatant are taken in new tubes and stored immediately at -80°C.

Conclusions (if applicable)

Homogenates should be used for analysis no later than 6 months after homogenization, because he activity of the analyzed enzymes starts to decrease in long term storage.

References

[1] Turja, R., Höher, N., Snoeijs, P., Baršienė, J., Butriavičienė, L., Kuznetsova, T., Kholodkevich, S.V., Devier, M.H., Budzinski, H., Lehtonen., K.K., *Sci Total Environ 473-474,* **2014**, 398-409.

[2] Leiniö S., Lehtonen K.K., *Comp Biochem Phys C 140,* **2005,** 408-21.

Change history

1.0 12.2.2020 First edition

1.1 8.6.2020

List of authors

Aino Ahvo (1.0, 1.1)

List of Reviewers

Daniel Koske (1.0)

name (version)