Recommended Operating Procedure (ROP)

Aim of ROP (tick box)

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

16. Superoxide dismutase activity

version 1.1

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Scope

This ROP describes the analysis method for assessing oxidative stress in biological samples exposed to hazardous substances, including substances derived from sea-dumped munitions. Superoxide dismutase (SOD) is a major enzyme in the antioxidant defence system (ADS) functioning against reactive oxygen species (ROS). SOD catalyzes the conversion of the superoxide anion $O^{2-\cdot}$ to the less harmful hydrogen peroxide (H_2O_2), which is further broken down by catalase (CAT) and glutathione peroxidase (GPx). The method uses a commercial kit[1] 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)[2].

Summary of the method/ROP

Superoxide dismutase activity is measured spectrophotometrically from tissue homogenates of mussel digestive gland or fish liver. Measurements need to be done in samples from both the suspected dumping site and a reference area.

Safety aspects

Normal laboratory safety rules should be applied.

Documentation

All samples should have a unique code and label. Pipetting schemes should be used to record the analysis procedure and order of samples, as well as possible pipetting errors or other anomalies that could affect the interpretation of the results.

Absorption of each sample and replicate at 450 nm [OD/min]
Sample volume in the analysis [µl]
Protein concentration [mg/ml]
SOD activity [U mg^{-1} protein]

Methods

Matrix: Fish liver, mussel digestive gland and gill tissue homogenates (see SOPs for homogenisation[4,5]).

Equipment: Spectrophotometer/microplate reader able to measure at 450 nm; basic laboratory equipment (pipettes, 96-well microplates, decanters). For reagents, see Table 1.

Table 1: Reagents used in the analysis.

reagent/ solution	**details**	**CAS-number(s)**
SOD standard stock	30 U/ml dissolved in buffer	9054-89-1
SOD commercial kit	Sigma-Aldrich product #19160	

Measurements and units: Measurement of SOD activity is recommended to be performed with a commercial kit, e.g., Sigma-Aldrich 19160 SOD Determination Kit[1]. Make SOD standard dilutions from the stock according to Table 2.

Table 2: Standard curve.

code	SOD stock µl	buffer µl	final SOD concentration U/ml
P0	0	50	0
P1	8	42	4.8
P2	17	33	10.2
P3	25	25	15
P4	33	17	19.8
P5	42	8	25.2
P6	50	0	30

Pipette 5 µl/well of samples, standards and 2 blanks (buffer) in triplicate. Add WST working solution (from kit) 50 µl/well. Tap or centrifuge the plate to ensure mixing of the reagents. Measure the absorbance at 450 nm. Add enzyme working solution (from kit) 5 µl/well, except for one of the blank triplicates. Mix the plate with e.g. a shaking program of the spectrophotometer. Incubate 20 min at 37 °C. Measure the absorbance at 450nm. Sample volumes in the analysis can and should be adjusted to achieve a satisfactory result, i.e. in the range of the standard curve.

Calculations are done according to the kit protocol for a non-kinetic measurement. Briefly, subtract the absorbances measured before adding the enzyme working solution from the final absorbances. Plot a linear regression curve from the standards with the absorbances $\log_{10}$-transformed, and calculate the activity of SOD in samples based on the curve. The activity of SOD (Units/ml) is adjusted to the protein concentration of the sample, measured with e.g. the Bradford method[3].

Sample size: Measurements are made from at least 15-20 individual specimens from each study site.

Conclusions (if applicable)

SOD is ubiquitous in the animal kingdom and can be measured also in other species than those mentioned in this ROP.

Compare the SOD activity levels measured from organisms collected from the target area to those from the reference area. An elevated or lowered activity level (bell-shape response) compared to the reference area indicate a negative effect. If the difference in mean activity level is more than one standard deviation (SD) of the mean values measured in the reference area, stress is considered moderate. If the level differs more than two SDs, stress is severe.

References

[1]Sigma-Aldrcih, SOD Determination Kit Product Information, *Sigma-Aldrich Co, St. Louis,* ***2014***.

[2]Ahvo, A., Lehtonen K.K., Lastumäki A., Straumer K., Kraugerud M., Feist, S.W., Lang T., Tørnes J. *Marine Environmental Research,* **2020,** in revision.

[3]Bradford, M.M., *Anal Biochem 72,* ***1976****, 248-254.*

[4]Ahvo, A. *DAIMON 2 PROJECT SOPs:* Homogenisation of fish liver and mussel digestive gland tissues **2020.**

[5]Ahvo, A. *DAIMON 2 PROJECT SOPs: Homogenization of fish muscle and mussel gill tissues,* **2020.**

Change history

1.0	11.2.2020	First edition
1.1	12.6.2020	
1.2	18.5.2021	Definition of the document was changed from SOP to ROP.

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