NO_2^- assay using 2,3 – DIAMINONAPHTALENE (DAN) on *Aiptasia pallida*

(adapted from : Nussler et al. (2006). Fluorometric measurement of nitrite/nitrate by 2,3-diaminonaphthalene. Nature protocols *1*, 2223-2226.)

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Date : March 2009

Anemones harvest

Anemones are harvested after treatment and rinsed in a large volume (100 ml) of 37 mM $MgCl_2$ just before being flashed frozen in liquid nitrogen and stored at -80 °C.

Note : try to dry out as much as possible anemones before freezing. I used blot paper and it worked fine

Anemones grinding

Anemone are thawed on ice, then grinded in 340 µL of deionized water at 4°C. The solution is centrifuged at 14,000 g during 10 min at 4°C.

After centrifugation, 5 µl of the supernatant is used for protein quantification (Bradford assay). The remaining supernatant is harvested and poor on a CENTRICON column (10 KDA) pre-washed 3X with 500 µl of deionized water. The column is centrifuged at 14,000 g during 10 min at 25 °C. The filtrates (samples) are conserved at -20°C.

NO_2^- assay

The samples are thawed at room temperature.

A standard curve is build up from $NaNO_2$ solution 1mM conserved at 4°C during maximum 8 weeks. The concentrations for the standard curve in duplicate are 10,000 ; 5,000 ; 2,500 ; 1,250 ; 625 ; 312 ; 156 nM.

In a 96-well black plate for fluorescence reading (with no autofluorescence- NUNC cat # 437112), 150 µl of standard or sample are added per well. 75 µl of a DAN solution at 158 µM/HCl 0.62 N is added to each well. This DAN solution can be conserved at 4°C during 4 weeks into the dark. Mix the plate during 10 sec and incubate at 28 °C into the dark during 10 minutes before adding 35 µl of a 2 N NaOH solution. Incubate 10 min into the dark.

Read the emitted fluorescence at 450 nm after excitation at 365 nm.