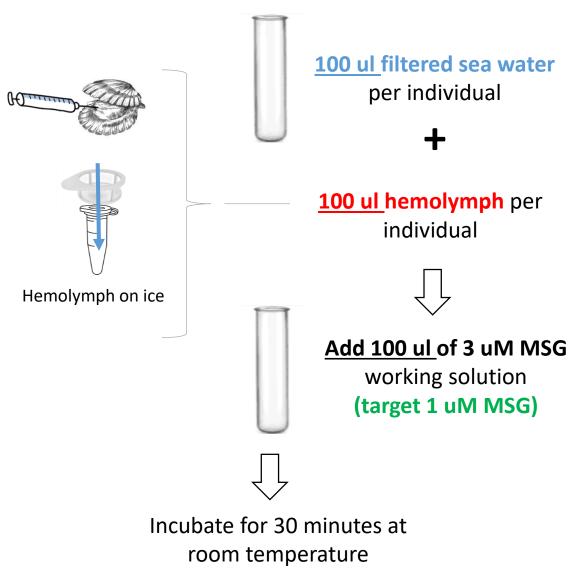
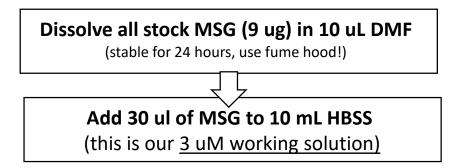
Flow cytometry: MitoSox Green Overview



Measure on flow cytometer

Make 3 uM working solution of MitoSox green!



NOTE! The volume of hemolymph will dictate how much 3 uM MSG working solution we add.

For example, if we have **only 50 ul hemolymph**... add 50 ul FSW and **add 33.33 uL 3uM MSG** for 1uM!

Flow cytometry: <u>JC-10</u> & <u>JC-10</u> + <u>CCCP</u> *Overview* 100 ul filtered sea water per individual **100 ul hemolymph** per individual Hemolymph on ice Add 15 ul of 200 uM JC-10 working solution (target 15 uM JC-10) Control! Incubate for 30 minutes at Add 2 uL of room temperature 1 mM CCCP (target 10 uM CCCP) Measure on flow cytometer

Make 200 uM working solution of JC-10!

NOTE: this targets 15 uM JC-10 in a 200 uL sample

JC-10 stock is 3428uM,

To make sufficient 200 uM working solution

for 45 samples.. (actually 90 including the control!)

Add 81.69 ul JC-10 stock with 1,318.3 uL HBSS

NOTE! The volume of hemolymph will dictate how much 200 uM JC-10 working solution we add.

For example, if we have **only 50 ul hemolymph**... add 50 ul FSW and **add 7.5 uL 200 uM JC-10** for 15 uM

Make 1 mM working solution of CCCP!

NOTE: this targets 10 uM CCCP in a 200 uL sample

Our CCCP is 100 mg of 98% in solid form, molecular weigh is 204.62 g/mole

Dissolve all CCCP in **48.8 mL HBSS** in a labeled bottle, **this is 1 mM CCCP working solution**

NOTE! if we have **only 50 ul hemolymph**... add 50 ul FSW and **add 1 uL 1 mM CCCP** for 10 uM

Flow cytometry: **SYBR Green + Propidium iodide** *Overview*

