

## Drinks Like a Fish – Student Handout #3

### Are the Toxic Effects of Alcohol Due to Oxidation?

Instructions for dilutions of ethanol and nicotinamide.

#### 1. **Make a stock solution of ethanol (10%)**

Depending on the concentration of ethanol you have on hand (e.g., 100%, 95%, or 70%), make a 10% stock solution in egg water. Make about 10 mls. Use the table below to calculate how much of each reagent to use. The first line is filled in for you if you are starting with 100% ethanol.

Starting ethanol concentration	# of mls of ethanol	# of mls of egg water	total # of mls
100%	1 ml	9 mls	10 mls
95%	1 ml		
70%	1 ml		

#### 2. **Make a stock solution of the antioxidants nicotinamide (500 mM).**

First determine the molecular weight (MW) of the compound, the proposed concentration for treatment, and whether the compound is soluble in an aqueous (i.e., water) or organic solvent (i.e., DMSO).

Nicotinamide MW: \_\_\_\_\_

Nicotinamide Solubility: \_\_\_\_\_

How many grams of nicotinamide do you need to make a 500 mM solution in 10 mls?

Your teacher will weigh out the appropriate amount and dissolve in 10 ml water.

#### 3. **Make working solutions.**

0.2 % ethanol (in egg water)

Stock ethanol concentration	Working ethanol concentration	# of mls of stock ethanol	# of mls of egg water	total # of mls
10%	0.2%			100 mls

5 mM nicotinamide (Nic) (in egg water)

Stock Nic concentration	Working Nic concentration	# of mls of stock Nic	# of mls of egg water	total # of mls
500 mM	5 mM			100 mls

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### 5 mM nicotinamide (Nic) (in 0.2% ethanol)

Stock Nic concentration	Working Nic concentration	# of mls of stock Nic	# of mls of 0.2% ethanol	total # of mls
500 mM	5 mM			100 mls

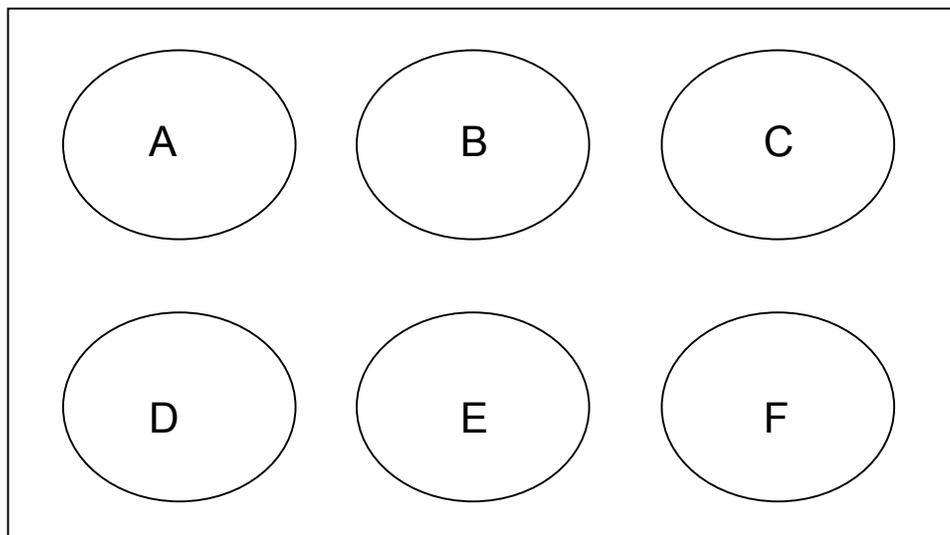
It's best to prewarm the solutions to 27°-28° C if you are doing the entire experiment at that temperature. If not, just use room temperature.

### Set-up your treatment plates (6-well plates)

1. Take a clean 6-well plate, labeling the lid with code letters according to the chart below (A-F). You have 4 treatment conditions: no ethanol (control), 0.2% ethanol, 5 mM nicotinamide, and 5 mM nicotinamide in 0.2% ethanol. There are enough treatment wells to do duplicate the two ethanol-exposed conditions. Doing duplicate conditions increases your accuracy, and is helpful in case you make a mistake.

Well Label	Solution for Experimental Treatment
A	Control – Egg water alone
B	0.2% Ethanol (made in egg water)
C	5 mM Nicotinamide in egg water
D	5 mM Nicotinamide in 0.2% ethanol
E	Duplicate of Well B
F	Duplicate of Well D

2. You can label the wells as follows (use a “Sharpie”)  
Label the net-inserts the same way.



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3. Add 2 mls of the corresponding solutions (pre-warmed) to each well (use your table above)
4. Add the net-inserts into the wells
5. Add the remaining 7 mls of solution into the net well-insert (this helps avoid air bubbles)

### Expose the 4 day old zebrafish to the treatment solutions

1. Transfer 10 zebrafish into the net well-inserts containing 9 mls of prewarmed egg water (with or without alcohol or nicotinamide) using a pipette
2. Place the lid on the plate and then wrap the plate with parafilm or plastic wrap.
3. Put plates in incubator/oven at 27°-28° C for 18-24 hours. If you incubate at room temperature, it will take longer to produce the neuromast damage.
4. The next day, fill 3 more 6-well plates with fresh egg water at 27°-28° C (9 mls/well). Be sure the net wells themselves are labeled and not just the 6-well plate!! Transfer the net wells (individually) into one of the new plates containing fresh egg water. Allow them to sit for 5 minutes. Repeat two more times. This is a “wash” step and ensures that no residual ethanol remains.
5. Your fish are now ready to be tested in the super-duper acoustic startle apparatus. Go to Student Handout #2.

### **Attention!!**

Before doing any testing, you need to be “blinded” by the treatment well. Your teacher will reassign the well designations with new letters and keep the “code” secret until your experiment is over. This will avoid any bias on your part while you are giving your fish their movement scores. After the experiment is over, your teacher will “break the code” and you can write the actual treatment into your data column.