

## Protocol for 4OHT in CDH5 cre mice

\*4-OHT is purchased from Sigma (Cat. No. H7904-25MG)

1) Prepare a 20 mg/mL stock in 100% EtOH, which has to be heated for ~10 min at 60 degrees and, if possible, sonicated in a bath sonicator briefly (~ 10-20 seconds).

Prepare 50 ul aliquots of this **20 mg/mL stock** and store in the freezer (-20).

2) When you're ready to inject, thaw a 4-OHT aliquot at 65 degrees for 10 min, vortex every 2-3 min and spin down with a microcentrifuge after each vortex. It's normal to see precipitate before heating.

During this time, warm up corn oil to at least 37 degrees (65 degrees is better).

3) Add warm corn oil to the 4-OHT/EtOH for a final concentration of **2 mg/mL 4-OHT** (dilute 4-OHT 10-fold). If I am not mistaken, the final concentration of EtOH will be 10%.

Heat this at 37 degrees (with agitation, if possible) for 45 min and vortex/sonicate every 15 min.

4) We inject 30 ul of the 2 mg/mL 4-OHT solution for 60 ug total per pup aged P1-P3. The dose is 40 mg/kg.

We inject subcutaneous, under the neck/back skin. We typically see that ~10-20% of the oil leaks out of the skin after injection. It's best to hold the needle under the skin for ~20 seconds, then slowly pull the needle out.