

Fly Ethanol Treatment Protocol

Susan-Celniker lab

Protocol reference: Morozova, Anholt and Mackay 2006

Adult Ethanol treatment:

Set up an ethanol treatment chamber where compressed air is bubbled through 95% ethanol and the vapor is passed through a tube containing the flies. Transfer adult flies to the treatment chamber and expose to concentrated ethanol vapor for 5 minutes. Return flies to standard fly media for 2 hours. Return flies to treatment chamber and expose to concentrated ethanol vapor for 8.5 minutes. Recover flies and immediately snap freeze flies in liquid nitrogen.

Larval ethanol treatment:

From Steve McKechnie (pers. comm.): "My inclination would be to put 3rd instars onto 2.5% ethanol – a high 'natural' level that is probably 'a rich feed', and well above zero levels that I suspect is also common in natural habitats. I think 2.5% is likely to invoke a significant transcriptional change. However this might not be considered a stress level - to stress them I would go for 5% EtOH. Three hours in vials at these levels should do the trick. Keep in mind that often Nipagin is put into standard media and is often predissolved in EtOH." Prepare standard media. Add 5% (v/v) ethanol and mix. Transfer third instar larvae to media supplemented with 10% ethanol. Allow larvae to feed for 3 hours. Recover larvae and snap freeze immediately on liquid nitrogen.

Take note of the following table concerning % lethality of Ethanol:

Treatment	Stage	% Lethality	Notes
10% EtOH	Larvae	2.6%	3 HR Feeding
2.5% EtOH	Larvae	4.7%	3 HR Feeding
5% EtOH	Larvae	2.7%	3 HR Feeding