SOP_MTL-1.9 Estradiol Water Preparation

A. Purpose: To provide instructions for the formulation of estradiol water be administered to rodents.

B. Scope: The estradiol water can be used as drinking water for all animal protocols that require estrogen supplementation in water.

C. Definitions:

CCM: Center for Comparative Medicine, Baylor College of Medicine

EtOH: Ethanol

SCIF: Special care instruction form

D. Materials and Reagents:

Name	Quantity	Catalog Number	Sterility status for use
1000 mL Bottle with twist cap	1	1395-1L, Corning	Sterile
1000 µL Pipette tips (+pipette)	1-3	1000 µL: 05-403-18, Eppendorf	Sterile
50 mL Conical tube holder	1	50-998-383, Fisher scientific	Sterile
Autoclaved sipper tops	1/bottle	Water feeding bottle caps provided by your animal facility	Sterile
Autoclaved water	200 mL/bottle	Drinking water used at your animal facility	Sterile
Autoclaved water bottles	1/cage	Water feeding bottles provided by your animal facility	Sterile
Ice pack	1	NA	Non-sterile
Rescue solution	As needed	https://rescuedisinfectants.com	Non-sterile
β-Estradiol in ethanol 1000X (8 mg/mL), 50 mL	1	SOP_MTL-1.8 Estradiol Stock Formulation	Sterile

E. References:

SOP_MTL-1.8 Estradiol Stock Formulation

F. Procedures:

General Considerations:

- In order to keep the water as sterile as possible all bottles will be filled inside the hood.
- Spray all supplies with Rescue prior to placing them into the hood.
- One standard dose (for a cage of mice) requires 200 µl of β-Estradiol stock per 200 mL (1:1000 dilution) of water for a final concentration of 8 µg/mL.

- If more than one person is going to be filling water bottles, bring a 1000 mL bottle for each person.
- Per SCIF, red acetates or blue stickers on a cage indicate that rodents require estrogen water.
- This SOP is written in accordance with guidelines required for animal facilities at Baylor College of Medicine. Please follow your institutional guidelines for procedures regarding special water usage and identification of cages containing special water.

1. In the lab, place the β-Estradiol 1000X stock tube into a small bag with an ice pack to keep it cold. Take the 1000 mL bottles, wrap them in a cage cover cloth (to prevent them from breaking), and place them in a plastic bag.
2. In TMF, spray the plastic bags into the procedure room.
3. Remove all old estradiol water bottles from cages marked with red acetates, place them in an empty water bottle rack, and leave them on the dirty equipment rack located outside VD7.
4. Get the tips, pipette, and tube holder from the container in VD7 and place them in the hood.
5. Place the 50 mL tube of β-Estradiol 1000X stock and empty 1000 mL bottles in the hood.
6. Place empty water bottles in the hood and remove the sipper tops. Put the tops to the side for later use.
7. Using the autoclaved/chlorinated water bottles provided by CCM, pour 1000 mL of water into each 1000 mL bottle (up to the solder line). The empty water bottles can then be used to fill with β-Estradiol drinking water.
8. Add 1000 μL (1mL) of β-Estradiol 1000X stock to the 1000 mL bottle.
9. Screw on cap and gently invert to mix.
10. Into an empty water bottle (from step 6 or 7), pour 200 mL of β-Estradiol drinking water. Since the β-Estradiol drinking water is pre-mixed, this measurement does not have to be exact. 200 mL is usually about half of the water bottle. Use the measurement lines on the 1000 mL bottle and adjust accordingly.
11. Place sipper top back onto the water bottle and tighten.
12. Repeat until you have filled the desired number of bottles.
13. Place the E2 water bottles on to the cages marked with red acetates and push the cage back into the rack. Double check to make sure every cage has a bottle and that none are leaking.
14. Clean the hood and return all supplies and used equipment to the correct locations.
15. Take the β-Estradiol 1000X stock tube back to the lab and promptly return to -20°C freezer. Put the 1000 mL bottles in the dirty glassware container.

G. Revisions Log

Version	Revision Date	Section Revised	Notes
1	02.05.2021	All	SOP created