

SOP_MTL-1.8 Estradiol Stock Formulation

- A. Purpose:** To provide aseptic technique and instructions for the formulation of stock estradiol to be used in the preparation of estrogen water.
- B. Scope:** The estradiol stock can be used to prepare the drinking water for all animal protocols that require estrogen supplementation in water.

C. Definitions:

CV: Current Volume

EtOH: Ethanol

D. Materials and Reagents:

Name	Quantity	Catalog Number	Sterility status for use
1000 mL Bottle with twist cap	1	1395-1L, Corning	Sterile
1000 mL Erlenmeyer flask	1	4980-1L, Corning	Sterile
25 mL Serological pipette	2	P7865, Greiner	Sterile
50 mL Conical tubes	14	14-959-49A, Fisher scientific	Sterile
70% Ethanol spray bottle	1	LC222102, Fisher scientific	Non-sterile
Estrogen powder	1	β-Estradiol E2758-5G, Sigma	Sterile
Large stir bar	1	14-513-82, Fisher scientific	Sterile
Multi-size test tube rack	1	03-448-17, Fisher scientific	Sterile
Pipet-aid	1	4-000-101, Drummond	Sterile
Pure Ethyl Alcohol, USP grade	2	Ethyl Alcohol, Pure 493546-500 mL, Sigma	Sterile

E. References:

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F. Procedures:

General Considerations:

- In order to keep the solution as sterile as possible, mixing of reagents will be done inside the cell culture hood.
- Use aseptic techniques throughout the procedure.
- All items going into the cell culture hood should be sprayed with 70% ethanol and wiped down with a paper towel.

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1. Remove the foil from the Erlenmeyer flask and put the foil on the side. Label the flask “ β -Estradiol 8 mg/mL”.
2. Label the bottle with the twist cap “Pure EtOH” and open it. Then open the EtOH stock bottle and pour around 625 mL into the “Pure EtOH” bottle. Close both bottles. This will ensure that the stock EtOH bottle remains sterile.
3. Place a 50 mL conical tube onto the multi-size test tube holder. This will be used to hold the 25 mL serological pipette between EtOH additions.
4. Slowly open the bottle of estrogen as powder the powder is light and may easily float out.
5. Attach a 25 mL serological pipette to the pipet-aid and withdraw 25 mL of EtOH from the “Pure EtOH” bottle and dispense it slowly into the bottle of estrogen powder. Gently mix the solution by pipetting up and down. Detach the 25 mL pipette from the pipet-aid and place in the 50 mL conical tube.
6. Slowly pour the mixture from the estrogen powder bottle into the Erlenmeyer flask. [CV: 25 mL].
7. Now pipette a total of 50 mL EtOH, by pipetting out 25 mL of EtOH twice from “Pure EtOH” bottle, into the estrogen powder bottle. Mix it by pipetting up and down. Detach the 25 mL pipette from the pipet-aid and place in the 50 mL conical tube. Pour the mixture into the Erlenmeyer flask. [CV: 75 mL].
8. Repeat step 7 eleven times. The CV will now be 625 mL with a final concentration of 8 mg/mL.
9. Ensure there is no writing on the stir bar. Open the autoclave pack, slide the stir bar into the Erlenmeyer flask, and cover the top with the same foil that you removed in step 1.
10. Clean the cell culture hood with 70% EtOH. Dispose of glass in the broken glass box and dispose of plastic in the regular trashcan.
11. Lower the cell culture hood sash and turn on the UV light.
12. Take the Erlenmeyer flask to the chemical hood and place it on the stir plate inside the hood.
13. Turn the stir dial to 7 or 8; make sure the stir bar is spinning rapidly. Make sure the heat is off.
14. Let the solution stir for 1-2 hours.
15. Label thirteen 50 mL tubes with “ β -Estradiol in Ethanol 1000X (8 mg/mL)” and the date, in order to make 50 mL aliquots.
16. After the solution has finished stirring, take the Erlenmeyer flask to the cell culture hood along with the 13 labeled conical tubes.
17. With a 25 mL serological pipette, fill each labeled conical tube with 50 mL of stock estrogen. The last tube will only have around 25 mL.
18. Store the tubes in the -20°C freezer #3.

G. Revisions Log

Version	Revision Date	Section Revised	Notes
1	02.05.2021	All	SOP created