May 01, 2025

Tamoxifen Administration for Aldh1l1-CreERT2, Drp1 flox mice

The Journal of Cell Biology

DOI
https://dx.doi.org/10.17504/protocols.io.dm6gp21b5lzp/v1

Pia Rodriguez[1]

[1]Duke University

Pia Rodriguez
Duke University

DOI: https://dx.doi.org/10.17504/protocols.io.dm6gp21b5lzp/v1

External link: https://doi.org/10.1083/jcb.202410130

Protocol Citation: Pia Rodriguez 2025. Tamoxifen Administration for Aldh1l1-CreERT2, Drp1 flox mice. **protocols.io** https://dx.doi.org/10.17504/protocols.io.dm6gp21b5lzp/v1

Manuscript citation:
Salazar MPR, Kolanukuduru S, Ramirez V, Lyu B, Manigault G, Sejourne G, Sesaki H, Yu G, Eroglu C (2025) Mitochondrial fission controls astrocyte morphogenesis and organization in the cortex. The Journal of Cell Biology 224(10). doi: 10.1083/jcb.202410130

Protocol status: Working
We use this protocol and it's working

Created: April 30, 2025

Last Modified: May 01, 2025

Protocol Integer ID: 203437

Keywords: Tamoxifen, drp1 flox mice, aldh1l1, creert2, mice

Funders Acknowledgements:
HHMI Gilliam Fellowship
Grant ID: GT15076
Paul and Daisy Soros Fellowship
Chan Zuckerberg Initiative, Neurodegeneration Challenge Network Collaborative
Grant ID: DAF2018-191999
Chan Zuckerberg Initiative, Neurodegeneration Challenge Network Collaborative
Grant ID: DAF2021-237435
BRAIN Initiative
Grant ID: U19-NS123719

Abstract

Adapted from Nature Protocols 2010 Pitulescu et al and Marchuk Lab

Guidelines

ALL TAMOXIFEN WORK SHOULD BE DONE IN A FUME HOOD. MATERIALS ARE TO BE DISPOSED OF IN BIOHAZARD WASTE AND PLACED IN HOOD UNTIL ARRANGED FOR PICK UP.

Materials

Tamoxifen
Corn Oil
Insulin Syringes

Prepare Tamoxifen Stock Solution (10 mg/mL, make 1-2 days before injection day)

1 Add 10 mg Tamoxifen to 1 mL corn oil.

2 Seal the tube with parafilm. Wrap the tube in aluminum foil to keep light protected. Add tape to the tube and label as 'Tamoxifen'. Bring the vortex into the fume hood. Mix the tube well by vortexing briefly and then incubate at 37°C overnight by placing the wrapped tube on the nutator in theincubator.

3 The next day, confirm the solution is properly homogenized. Store your stocks at -20°C for up to 3 months light protected. All stocks should be well labeled as 'Tamoxifen' and stocks stored long term should be kept in a small biohazard bag that is thoroughly labeled as tamoxifen.

Prepare Tamoxifen working stock solution (2.5 mg/mL, make day of first injection)

4 Will need 80 uL total of working tamoxifen solution per pup for all 3 days, round up to 100 uL total. Therefore, after litter drops, count the number of pups and make the appropriate amount of working solution.

4.1 For a 10-pup litter: Add 250 uL of tamoxifen stock solution (10 mg/mL) to 750 uL corn oil. Mix well by pipetting up and down in the fume hood. Try to avoid making bubbles. If there are air bubbles, place in the 37°C nutator in the incubator for ~30 minutes.

5 Keep working stock at 4°C for up to 5 days protected from light wrapped in aluminum foil in a box clearly labeled tamoxifen with the date working stock was made.

6 After 5 days or after you are done injecting, all other Tamoxifen solid waste should be placed in a biohazard waste bag in the fume hood.
Any left-over stock older than 5 days should be injected into a mouse carcass and can be discarded as normal (green mouse carcass bag).

Injection Protocol

7 On injection day, take the following with you to the mouse house:
- Box with tamoxifen working stock tube wrapped in aluminum foil
- Clean small biohazard bag for tamoxifen waste
- Sheet to document injection notes for each pup
- Insulin syringes
- Tubes to collect toes

8 Once at the mouse house, you will need two sets of gloves. After you are done with the injections, you should remove the top layer of gloves and dispose of these and any tamoxifen plastic waste into a small biohazard bag (brought from the lab into the mouse house). Needles can be disposed of in the regular sharps disposal.

9 Perform first tamoxifen injection no earlier than the afternoon (3 pm or later) of P1.

10 Pups will be injected into the milk spot with 50 ug tamoxifen on 3 consecutive days.

Day 1-2: 20 uL of 2.5 mg/mL = 50 ug total dose per day (1 and 2). Day 3: 40 uL of 2.5 mg/mL = 100 ug total on day 3.

11 Use small insulin syringe. Be sure to inject 20 uL on days 1-2 and 40 uL on day 3.

12 Pups should be toe clipped on the same day as the first Tamoxifen injection to keep notes on each individual mouse regarding the injections.

13 Return the pups to the mom. Supplemental care can be given to try and offset stress on the mom.