

MUTANT MOUSE REGIONAL RESOURCE CENTER: UC DAVIS

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Tamoxifen Preparation and Oral Dosing of Adult Mice

Protocol provided by GENSAT

1.0 Scope:

- 1.1 To describe the procedure for preparing tamoxifen and dosing an adult mouse orally with tamoxifen for five consecutive days.

2.0 Materials:

- 2.1. Tamoxifen, Minimum 99% (Sigma Cat# T5648-5G)
- 2.2. Corn oil (Sigma Cat# C8267-500ML)
- 2.3. 8ml Amber glass vial
- 2.4. Disposable 1/2 x 1/8 in magnetic stir bar
- 2.5. Aluminum foil
- 2.6. 1ml syringe
- 2.7. 24G gavage needle 1¼ mm ball
- 2.8. Eppendorf 1000uL pipettor
- 2.9. 1000uL tip
- 2.10. Metal spatula
- 2.11. DietGel R/E

3.0 Equipment:

- 3.1. Thermo controlled magnetic mixer hotplate
- 3.2. Mettler Toledo AB204s Analytical Scale in the Atlas Lab
- 3.3. Supreme Air fume hood in Atlas lab
- 3.4. 4°C fridge

4.0 Safety:

- 4.1. Universal Precautions should be used when handling animals and tamoxifen. The PPE required is a disposable lab coat, gloves, and eye protection.

5.0 Output:

- 5.1. 50mg/ml solution of tamoxifen is created and administered orally to mice over a five day period in order to induce Cre.

6.0 Setup:

- 6.1. Fill out a sheet to track the animals to be dosed over the next five days.
- 6.2. Weigh the mice needing to be dosed and use the table below to determine the amount of tamoxifen to be given over the next five days. Write both the weight of the mouse and the corresponding volume of tamoxifen solution on the sheet.

Weight of Mouse (up to)	Volume of Tamoxifen Solution (50mg/ml)
10g	0.04 ml
15g	0.06 ml
20g	0.08 ml
25g	0.10 ml
30g	0.12 ml
35g	0.14 ml
40g	0.16 ml

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- 6.3. Calculate the volume of tamoxifen that needs to be prepared by multiplying the amount of tamoxifen needed per mouse per day by five.

e.g.

22g mouse	→	0.10ml tamoxifen	*	5	=	0.50ml tamoxifen
20g mouse	→	0.08ml tamoxifen	*	5	=	0.40ml tamoxifen
Total					=	0.90ml tamoxifen

- 6.4. Add an additional 0.5ml to the total to account for dead volume in the syringe and any solution that may be lost during dosing.

- 6.5. Determine the exact amount of tamoxifen crystals needed

e.g.

$$\frac{X\text{mg}}{1.40\text{ml}} = \frac{50\text{mg}}{\text{ml}} \rightarrow \frac{70\text{mg tamoxifen}}{1.4\text{ml oil}}$$

- 6.6. Obtain bottle of tamoxifen from desiccator in 4°C fridge.
- 6.7. Bring tamoxifen, metal spatula, and glass vial to the Atlas lab. Measure out the desired amount of tamoxifen into the vial using the Analytical Scale under the Supreme Air fume hood.
- 6.8. Add the appropriate amount of oil using the pipettor to create the 50mg/ml solution.
- 6.9. Set temperature of the mixer hotplate to 40°C and place vial on the hotplate covered with foil to protect the light-sensitive tamoxifen solution from light exposure. Let mixture spin at 760 RPM for ten minutes, check to see if all the tamoxifen crystals have been dissolved before using, and allow it to spin for longer if necessary.
- 6.10. Wrap vial in foil to protect from light.

7.0 Methodology:

- 7.1. Pull the cage(s) containing the mice to be gavaged from the rack.
- 7.2. Pick the first mouse listed on the sheet and record the time, observations of the health of the mouse, and your initials.
- 7.3. Fill the syringe past what you need with 50 mg/ml tamoxifen.
- 7.4. Fit the gavage needle onto the syringe and push the plunger until tamoxifen is released from the end of the gavage needle and the level is at the appropriate marking.
- 7.5. Restrain the mouse and gently guide the needle down the esophagus until the base of the needle reaches the mouth. If any resistance is encountered by the needle, retract and start again, do not push.
- 7.6. Dispense the tamoxifen and then slowly remove the needle. Place the mouse back into the cage.
- 7.7. Repeat steps 7.2 to 7.6.
- 7.8. Repeat steps 7.1 through 7.7 for four more consecutive days, inducing the mice at the same time each day.
- 7.9. On the fifth day, weigh the mice a final time to check that there was no significant weight loss during the dosing period and add a scoop of DietGel R/E to the cage(s).

8.0 Take Down

- 8.1. Store remaining tamoxifen for up to a week in the fridge at 4°C.
- 8.2. Aspirate water through the syringe three times and 70% ethanol three times. Attach gavage needle to an empty plastic syringe and blow a puff of air through to remove excess ethanol.
- 8.3. Place gavage needle to dry on blue absorbent pad to dry and toss used syringe in the biohazard trash.

9.0 Disposal

- 9.1. Dispose of the glass vial, including the disposable stir bar and any remaining tamoxifen in the sharps container when done with the tamoxifen or after a week from preparation date.

***Note:** We start tamoxifen treatment for adult mice around six weeks of age. Older mice can be used as long as the amount of tamoxifen is adjusted for weight. We dose for one week, and then allow one week recovery time prior to dissecting; so, dose days 1-5, rest days 6-14, and dissect any day 15-20.