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## Tamoxifen Administration to Mice

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### Abstract

The strategy of fusing a protein of interest to a hormone-binding domain (HBD) of a steroid hormone receptor allows fine control of the activity of the fused protein. Such fusion proteins are inactive in the absence of ligand, because they are complexed with a variety of intracellular polypeptides. Upon ligand binding, the receptor is released from its inhibitory complex and the fusion protein becomes functional. In the murine estrogen receptor (ER) fusion system, proteins are fused to the HBD of the ER. The system relies on the use of a mutant ER known as ER<sup>TAM</sup>. Compared to the wild-type HBD, ER<sup>TAM</sup> has 1000-fold lower affinity for estrogen, yet remains responsive to activation by the synthetic steroid 4-hydroxytamoxifen (4-OHT). Because 4-OHT is expensive, animals can be treated with the cheaper precursor tamoxifen, which is converted into 4-OHT by a liver enzyme. Here we present an overview of the methods used to deliver tamoxifen to mice. The most used method is intraperitoneal injection, because the amount of administered compound can be better controlled, but delivery by oral gavage is also possible. For short-term and immediate-effect studies or when conversion of tamoxifen by the liver is to be avoided, 4-OHT can be used directly.

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### Materials

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

### Reagents

Distilled water

Ethanol

Ligand (Tamoxifen Powder [Sigma-Aldrich] or 4-hydroxytamoxifen [4-OHT] powder [Sigma-Aldrich])

Mice that express fusion proteins containing ER<sup>TAM</sup>

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For a discussion of the development and utilization of the ER-fusion system in mice, see Introduction: The Estrogen Receptor Fusion System in Mouse Models: A Reversible Switch (Whitfield et al. 2014).

Peanut or corn oil

## Equipment

Needles (21-gauge 5/8 for intraperitoneal injection or 22-gauge feeding needle for gavage)

Pestle for 1.5-mL tubes

Rotating wheel

Syringes (1 mL)

Tubes (15 or 50 mL)

## Method

1. Prepare the ligand.
  - For injection of tamoxifen, suspend tamoxifen powder in peanut or corn oil to a final concentration of 10 mg/mL.

Note that tamoxifen does not readily go into suspension. We recommend using a pestle to accelerate its resuspension or placing the tube on a rotating wheel overnight. The tube should be protected from light.
  - For injection of 4-OHT, dissolve 100 mg of 4-OHT powder in 1 mL of ethanol (final concentration of 100 mg/mL), and then dilute the solution 1/10 in peanut or corn oil.
2. Aliquot the solution in 1.5 mL tubes and store at  $-20^{\circ}\text{C}$ .
3. Before injection, thaw the required aliquots and bring them to room temperature or, preferably, warm them up to a temperature close to that of the mouse body.
4. Inject the animals.
  - To administer tamoxifen or 4-OHT intraperitoneally, use a 1 mL syringe and a 21-gauge 5/8 needle (100  $\mu\text{L}$  to inject 1 mg). Localize the needle in the lower abdomen to ensure you do not puncture any internal organ.

See Troubleshooting.
  - To inject tamoxifen by oral gavage, use a 1 mL syringe and a 22-gauge feeding needle (100  $\mu\text{L}$  to inject 1 mg). Place the mouse with its belly on a surface and restrain at its neck, tightly enough to keep its head immobilized but also to allow proper breathing. Gently introduce the

feeding needle in the mouth behind the tongue to a maximum depth of 1 cm, and deliver the required amount of solution.

## Troubleshooting

*Problem (Step 4):* In long-term studies, repeated intraperitoneal injection can result in the accumulation of oil in the peritoneal cavity and associated sterile peritonitis.

*Solution:* Administer tamoxifen in chow or drinking water (see Discussion).

## Discussion

To avoid difficulties associated with intraperitoneal injection, tamoxifen can be administered either into the food or the drinking water of the animal. To deliver in chow, use a commercially available tamoxifen formulation (dosage in this case depends on eating behavior). The standard concentration is 400 mg of tamoxifen citrate/kg of irradiated food pellet (Kiermayer et al. 2007). Note that mice may suffer a temporary loss of weight immediately after starting the new diet. To deliver tamoxifen in drinking water, dissolve it in pure ethanol (100 mg tamoxifen in 1 mL of ethanol) and then dilute it 100- to 200-fold in distilled water (to a final concentration of 1–0.5 mg/mL). Mice drink an average of 4–5 mL of water per day.

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