

Activation of ER-regulated Fusion Proteins *in vivo*

Jason Reuter

[Abstract] Regulatable protein fusions can be made by attaching the hormone binding domain of the estrogen receptor to the N or C-terminus of a protein of interest. Activation of such ER-fusion proteins *in vivo* can be achieved by daily administration of a synthetic ligand, 4-hydroxytamoxifen (4OHT), via intraperitoneal (i.p.) injection.

Materials and Reagents

1. Corn oil (Sigma-Aldrich, catalog number: C8267)
2. 200-proof ethanol (Sigma-Aldrich, catalog number: E7023)
3. 4OHT (Sigma-Aldrich, catalog number: H7904)

Equipment

1. Sonic dismembrator (Thermo Fisher Scientific, model: 100)
2. Centrifuges
3. Hybridization oven

Procedure

1. Dissolve the 4OHT in ethanol (67 mg/ml) by heating to 48 °C for 10 min.
2. Mix 4OHT solution with pre-heated (48 °C) sterile corn oil (7.5 mg/ml).
3. Sonicate 20 sec, at 5 volts then place on ice for 1 min. Repeat 3 times.
4. Aliquot solution to avoid freeze/thaw cycles and store at -20 °C for up to 1 month.
5. Prior to i.p. injection, 4OHT should be thawed, sonicated again (3x, 20 sec, at 3 volts) and injected immediately.

Note: If sustained activity is required daily injections must be performed.

References

1. Metzger, D. and Chambon, P. (2001). [Site- and time-specific gene targeting in the mouse. Methods](#) 24(1): 71-80.
2. Reuter, J. A. and Khavari, P. A. (2006). [Use of conditionally active ras fusion proteins to study epidermal growth, differentiation, and neoplasia. Methods Enzymol](#) 407: 691-702.