

Hormone/BrdU injection protocol

- Bed the animals on SaniChips, rather than corn pellets, to prevent any interference from phytoestrogens for 5-7 days before the hormone treatment.
 - Two days before sacrifice, inject mice with 100 µl of E+P under the skin between the shoulder blades. [The final dose given is 1 µg E₂ and 1 mg P]
 - Two hours before sacrifice, inject the mice with BrdU intraperitoneally (10 µl per gram of body weight....ie: 20 gram mouse would get 200 µl).
 - Remove the mammary glands, spread on parafilm paper squares, and place in tissue cassettes. Fix for 2 hours at 4°C in cold 4% paraformaldehyde.
 - After 2 hours, rinse the tissue with PBS and store in 70% ethanol at 4°C. The tissue could also be stained with Carmine Alum overnight at this point (see Carmine Whole mount protocol).
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Reagents:

Estrogen+Progesterone:

- β-Estradiol 3-benzoate, Sigma E-8515
[1,3,5[10]-Estratriene-3,17 β-diol 3-benzoate]
- Progesterone, Sigma P-0130
[4-pregnane-3,20-dione]
- Sesame oil, Sigma S-3547

E₂ stock: [10 mg/ml] stock in sesame oil
10 mg E₂ powder in 1 ml sesame oil.
Stir with mild heat (≤ 60°C) until dissolved.

E+P for injections: [10 µg/ml] E₂ Benzoate + [10 mg/ml Progesterone in sesame oil
100 mg progesterone powder
10 µl of [10 mg/ml] E₂ stock
10 ml sesame oil
Stir until dissolved.

BrdU: BromodeoxyUridine: Amersham RPN201 or
Sigma B-5002
Make up at [3 mg/ml] in PBS or saline. Store aliquots at -20°C.
Inject 10 µl per gram of bodyweight.

4% PFA:

1. Working in a fume hood, heat 450 ml of ddH₂O to 60°C.
2. With stirring, add 20 g of paraformaldehyde powder to the heated water. Cover and maintain ≤ 60°C on a hot plate.
3. Add 5 drops of 2N NaOH (1 drop per 100 ml). The solution should clear within a couple of minutes (There will be some fine particles that will not go away). **Do not heat solution above 70°C.**
4. Remove from heat and add 50 ml of 10X PBS. Adjust pH to 7.2; you may have to add some HCl. Filter and place on ice.