

## Rat Synchronization for Timed Pregnancy or Embryo Collection

### Materials:

1. SIGMA des-Gly<sup>10</sup>, [D-Ala<sup>6</sup>]-Leuteinizing Hormone Releasing Hormone Ethylamide Catalog number: L-4513 (5mg). (LHRH)- store at -20°C until re-suspended.
2. Dulbecco's Phosphate-Buffered Saline 1X (Gibco 14190-144). Store at room temperature.

### Resuspension of LHRH:

1. Make 200µg/mL stock by adding 25mL of Dulbecco's Phosphate- Buffered Saline 1X (PBS) to the 5mg vial of LHRH in the following manor:
  - a. Peel back foil cover on rubber stopper
  - b. Inject -1-2 ml of the PBS to the vial and mix well by rolling vial between the palm of your hands and tapping vial so that powder at top of vial goes into liquid. Continue mixing until all powder is dissolved.
  - c. Remove All of the liquid containing the hormone from the vial and transfer to a sterile glass cylinder. Add PBS until the volume is 25ml.
  - d. This should be performed in a laminar flow hood (Alternatively if sterile glass cylinders are not available- draw the liquid from the vial using a sterile 25 cc syringe with an 18G sterile needle and then draw up PBS until the final volume in the syringe is 25ml).
  - e. Aliquot in a laminar flow hood- 1mL per sterile tube or draw up 1ml into 1cc syringes and store in - 80°C for up to three months.
  - f. Record in hormone log.

### Procedure:

1. Inject Sexually Mature female rats (8 weeks of age -16 weeks best response – the younger animals generally give more embryos so 8-12 weeks of age is optimal ) on **Thursday the week before collection**. Ear tag females at the time of injection. Send a list of females injected and the strain to MU so that we can schedule collection for the following week.
2. Injections should be performed 2-3 hours after light induction (after the light turn on in the room for the day), give 40µg LHRH (.2mL) intraperitoneally (IP) per mature female rat. Use 1cc syringe with 26G 5/8 inch needle.
3. **On Monday-Four days after injection**, mate with a mature intact male rat (at least 10 weeks of age). Mate by moving the female to the male's cage in mid- to late-afternoon. Only put one female in with each male for optimal breeding results.
4. **On Tuesday morning**- record plug results for females and the id of the male the female was mated to.

