

## EGG COLLECTION

### Procedure:

1. Add 2-3 ml PLG media to 1 ml hyaluronidase (300 units/ml solution)
2. Add this to a 35 mm culture dish. Maintain at 37 C, 5% CO<sub>2</sub>.
3. Transfer egg masses directly into dish containing hyaluronidase.
4. Denuded eggs are transferred from the hyaluronidase dish into 2 wash dishes of PLG.
5. Washed eggs are placed into drops of PLG under silicone oil and maintained at 37C, 5% CO<sub>2</sub>.

### Reagent:

PVP 40,000 (Sigma P- )

Hyaluronidase Type IV from bovine testes (Sigma H-2376)

PBS

Dissolve 0.5 gm PVP and appropriate amount of hyaluronidase in 50 ml PBS. Filter sterilize. Dispense in 1 ml aliquots in Falcon 2063 tubes. Label with yellow tape. Store at -20°C.

Hyaluronidase final concentration is 300 units/ml. Example: if vial of hyaluronidase contains 1000 units/my, then add 15 mg in 50 ml.