

Early Hematopoiesis

Sterile bacteriological grade 100 x 20 mm petri dish

Sterile curved, blunt-ended, serrated forceps

Sterile curved, scissors

Sterile fine glass needle (5 3/4 inch Pasteur pipets)

Sterile 1.5-mL microfuge tubes

Paper rings cut from sheets of Whatman 3MM paper (inner ring dimensions 9 mm, outer ring dimension 13 mm; sterilize rings by gamma irradiation or by overnight exposure to UV light)

Pipete

Dissecting Microscope

Centrifuge

Methods:

- 1. Isolate blastoderm by cracking open the egg and pouring contents into sterile, bacteriological grade Petri dish.**
- 2. Remove the thick egg albumin with sterilized, curved, blunt-ended, serrated forceps.**
- 3. To harvest the embryo from the yolk**
 - a. place a paper ring over the blastoderm**
 - b. cut the adhered vitelline membrane around the ring with sterile, curved scissors**
- 4. Separate embryonic from extraembryonic tissue**
 - a. place paper ring with embryo on an inverted lid from a sterile, 35-mm Petri dish (make sure ventral side is facing up).**
 - b. remove any adhering yolk by gently pipeting with sterile, DPBS.**
 - c. keep the clean blastoderm in DPBS to maintain cell viability.**
 - d. using dissecting microscope, cut the area pellucida away from the area opaca using fine glass needles.**
 - e. cut area opaca and area pellucida into very small pieces. Take some of these for the cell dissociation study, but place others into drops fairly far apart of DPBS on an inverted lid from a sterile bacterial grade Petri dish.**

- f. place lid back on Petri dish, containing 2mL DPBS
(Note: make sure the drops do not merge with one another).
- g. incubate for 48 hours at 37 degrees Celsius with 5% CO₂. Make sure your dish is labeled.
- h. observe to see if any blood islands at start and end of incubation.

From Studying Early Hematopoiesis Using Avian Blastoderm Cultures. Eisenberg, Carol A. (pp 399-402). *In Methods in Molecular Biology, Vol. 137: Developmental Biology Protocols, Vol. III* Edited by: R. S. Tuan and C. W. Lo Human Press Inc., Totowa, NJ