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)
Pipette
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 sterilize, 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.