

Simple protocol for differential staining of inner cell mass and trophectoderm of bovine embryos

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This protocol is a slightly modified version of that described by Thouas et al. (Reprod Biomed Online 2001;3:25-29) and another one obtained from D.J. Walker and G.E. Seidel, Jr. at Colorado State University.

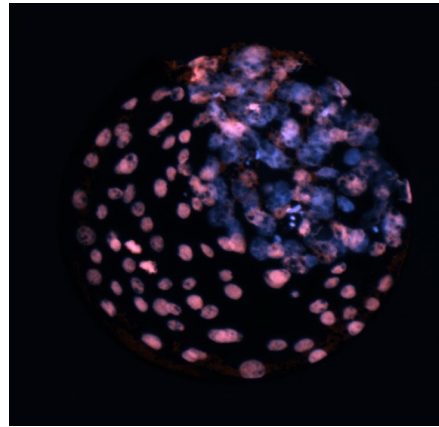
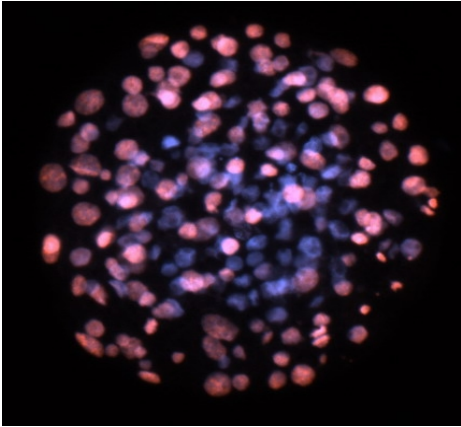
Materials

- 1) PBS/PVP – Add 1 mg/mL polyvinylpyrrolidone (**PVP**) to 0.1M PBS
- 2) 4% paraformaldehyde – Obtain 8% paraformaldehyde from Electron Microscopy Sciences (#15710-SP) and dilute 1:1 with PBS/PVP. Make fresh on the day of use and discard any leftover after using that day.
- 3) Triton X-100 – Dilute Triton X-100 in 0.1M PBS to obtain a final concentration of 0.5% (v/v).
- 4) Propidium Iodide (**PI solution**) - Prepare a 2.5 mg/ml stock 1 solution by dissolving PI (Sigma P4170) in PBS and store at 4°C. Next, dilute the stock 1 solution 1:25 in 0.5% Triton X-100 to give a working concentration of 100 µg/mL. Store stock 2 in a foil covered tube at 4°C for 3 weeks.
- 5) Hoescht 33258 (**H Solution**) - Prepare Stock 1 by dissolving 25 mg Hoechst 33258 (Sigma B2883) in 2.5 ml of distilled water (10 mg/ml). Store at 4°C. On the day of use, prepare Stock 2 by diluting the Stock 1 solution 1:1000 in 4% paraformaldehyde to give a working concentration of 1 µg/mL. Discard whatever is not used on the day of use.
- 6) Microscope slides – Fisherbrand Superfrost slides (#12-550-14).

Procedure

- 1) Place 500 µL of the PI solution in one well of a NUNC 4-well plate. Fill the remaining 3 wells with 500 µL of PBS/PVP.
- 2) Place the 4-well plate on a slide warmer set to 39°C and allow the PI solution to warm up for 5-10 min prior to beginning the staining procedure.
- 3) **TIP** - Conduct all following steps in a dark room to prevent bleaching. It may also help to cover the plate with foil.
- 4) Remove blastocysts from culture in as little medium as possible and place into the the well of PI solution for 30 seconds.
- 5) After the 30 second incubation in PI solution wash the embryos through the 3 wells containing PBS/PVP.
- 6) Place embryos into a 50 µL droplet of the H solution and incubate at room temperature for 15 minutes.
- 7) Wash embryos 3 times in PBS/PVP by moving them through 50 µL droplets.
- 8) Place embryos onto microscope slides in a small volume of solution. The place a small drop of glycerol over the embryos and mount coverslip. View embryos as soon as possible.

Representative Results



ICM are blue and TE are pink.