Differential Staining of Live and Dead Embryos using Fluoresce in Diacetate and Ethidium Bromide

J. Lannett Edwards[1] and P.J. Hansen[2]

[1]Animal Science-Experimental Station, University of Tennessee and [2]Dept. of Animal Sciences, University of Florida and

Procedures obtained from Ann Croy (Guelph, Canada) as a modification of procedures detailed in Transplantation 1971 12:148-151 Takasugi, M. An improved fluorochromatic cytotoxic test. Note that the procedure can be used for staining of all cells and not just embryos or oocytes. Also, ethididium bromide can be replaced with propidium iodide, DAPI or Hoescht 33342

Materials

Ethidium Bromide (EtBr; Fisher)
Fluorescein Diacetate (FDA; Sigma)
DPBS
Acetone

Procedure

1) Make following stock solutions:

   EtBr (10 mg/mL DPBS) Store in dark at 4 ☐C
   FDA (5 mg/mL acetone) Store in dark in glass container at -20 ☐C

   Storage life of stocks ~4 months

2) Just before use (i.e., ~10 min) prepare the following in a 15 mL conical tube covered with aluminum foil:

   100 µl EtBr Stock (0.05 mg/mL)
   3 µl FDA Stock (0.005 mg/mL)
   10 mL DPBS or culture medium

   Note: If you want to recover embryos from glass slide, use DPBS with 0.1% BSA or serum to prevent them from sticking to slide.

3) Place on ice until needed.

4) To stain embryos or oocytes place 50 µl of dye solution on a glass slide.

5) In the smallest volume possible transfer embryos or oocytes to be stained in the 50 µl and allow to sit in the dark for at least 3 min (FDA cleavage of acetate radical traps dye inside cell; 3 min=time for accumulation).

6) View embryos or oocytes for staining using the fluorescence microscope under UV epiluminesence (use UV filter).

   Live Stain=Green
   Dead Stain=Red/Orange

   Count green first before it "burns out" from illumination

Protocol created: February 2, 1995; online 02-21-2005, Last updated: February 22, 2005