Determining Lipid Content in Embryos using Nile Red Fluorescence

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The following protocol is derived from the protocol described by Genicot et al. (Theriogenology 63: 1181-1194, 2005) and is based on the fluorescence emitted by Nile Red when in association with lipid (see Fowler and Greenspan, J Histochem Cytochem 33: 833-836, 1985).

Equipments and Reagents

- **96 Well plate:** BD Falcon®,cat. # 353912, or equivalent
- **Nile Red:** Invitrogen Molecular Probles, cat. # N-1142
- **Hoescht 33342:** Sigma-Aldrich, cat. # B2261
- **ProLong® Gold antifage reagent:** Invitrogen Molecular Probes, cat. # P36934
- **Fluorescence microscope:** Fluorescent microscope configured with excitation 400-500 nm and emission 515 nm

Solutions

- **100 mM PO_4, pH7.4** (Stock solution)
  To prepare 100 mM PO_4 pH 7.4, add sodium phosphate monobasic (1a) to 300 ml sodium phosphate dibasic until the pH reaches 7.4. Approximately, 100 ml of monobasic solution will be added.
  1a. 100 mM sodium phosphate monobasic ($NaH_2PO_4.2H_2O$)
  13.8 g NaH_2PO_4
  1 L double–distilled (dd) ddH_2O
  1b. 100 mM sodium phosphate dibasic (Na_2HPO_4)
  14.2g $Na_2HPO_4.2H_2O$
  1 L ddH_2O
- **10 mM PBS/PVP** (Work solution)
  Dilute 100 mM PO4, pH 7.4 with 800 ml ddH_2O, add 9 g of NaCl and 1.0 g polyvinylpyrollidone (PVP), and bring volume to 1000 ml with water. Check pH.
- **4% Paraformaldahyde Fixative -** add 500 mL 8% (w/v) paraformaldehyde to 500 mL PBS-PVP (1:1 dilution)
- **Nile Red Stock Solution (1 mg/mL) -** dissolve 25 mg of Nile Red (Invitrogen N-1142) in 25 mL of DMSO; Store at room temperature in the dark indefinitely.
- **Nile Red Working Solution (1 µg/mL):** add 1 µL of Nile Red Stock Solution to 999 µL PBS-PVP; (*10 µg/mL is recommended by Molecular Probes but we prefer the lower concentration*).
- **Hoescht 33342 (optional to stain nuclei):** Prepare stock solution 1 by dissolving 25 mg Hoechst 33342 (Sigma B2261) in 2.5 ml double distilled water (10 mg/ml). Store in the dark at 4°C. On the day of use, prepare stock solution 2 by diluting 5 µl Stock 1 in 10 ml PBS-PVP to produce a 5 µg/ml solution. The working solution is prepared by diluting 200 µl of Stock solution 2 with 800 µl of PBS-PVP (final concentration 1 µg/ml).
- **Ethanol:** 100% Ethanol.

Procedure

- All procedures are done in a 96 well plate (Figure 1).
- Remove embryos from embryo culture and wash 3 times in 100 µL PBS-PVP (2 min for each wash).
- Transfer the embryos to 100 µL of Fixative. Wash two times in Fixative and incubate for at least 15 min.

- Negative Control: Before fixation, embryos can be incubated in 100 µL of 100% Ethanol for 30 minutes to dissolve lipid and create an embryo without green droplets (lipids) after completion of staining.
- Transfer the embryos to 100 µL of Nile Red Working Solution for 30 min.
- Wash embryos 3 times in PBS-PVP;
- Add embryos to slides in drops of ~ 1 µL of PBS-PVP containing one or more embryos, add 5 µL of ProLong® Gold antifage around the embryo and carefully place a cover slide on top of the drop.
- Lipid droplets can be visualized at 200x and 600x using a FITC filter; Nuclei counter-stained with Hoescht (optional) can be examined using a DAPI (UV) filter (UV)

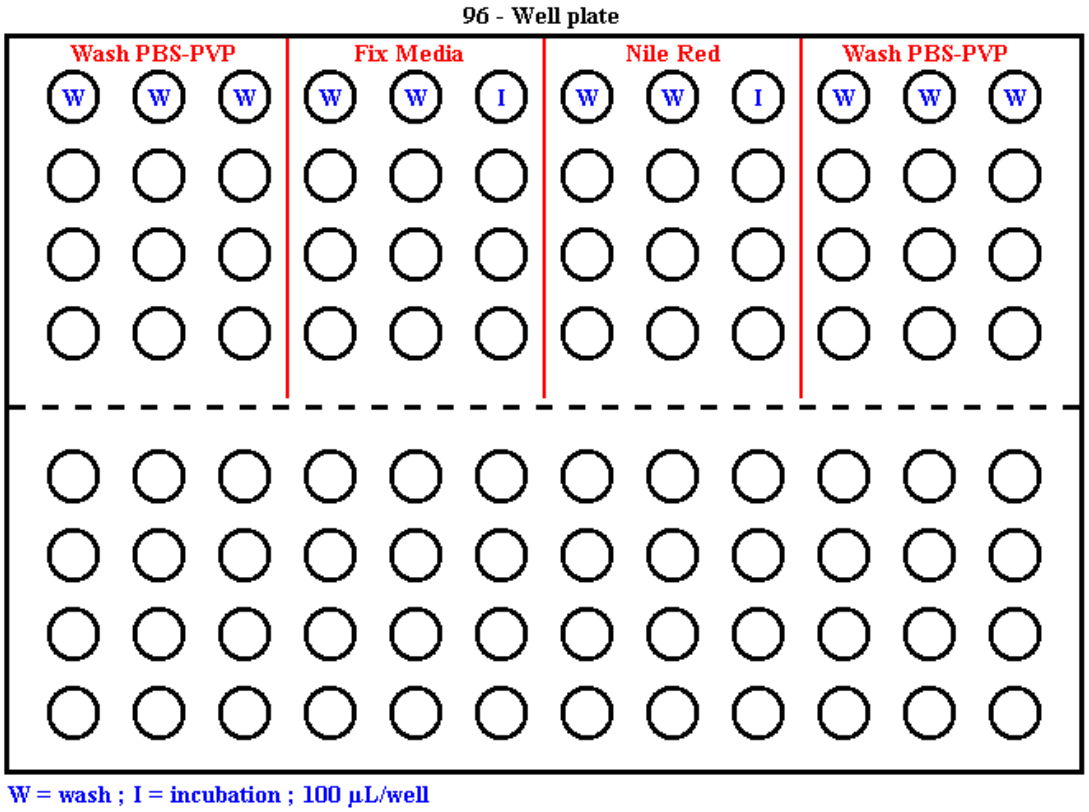

Figure 1. Arrangement of solutions to facilitate staining of embryos (fix media=fixative). Transfer embryos from well to well according to the procedures indicated above.

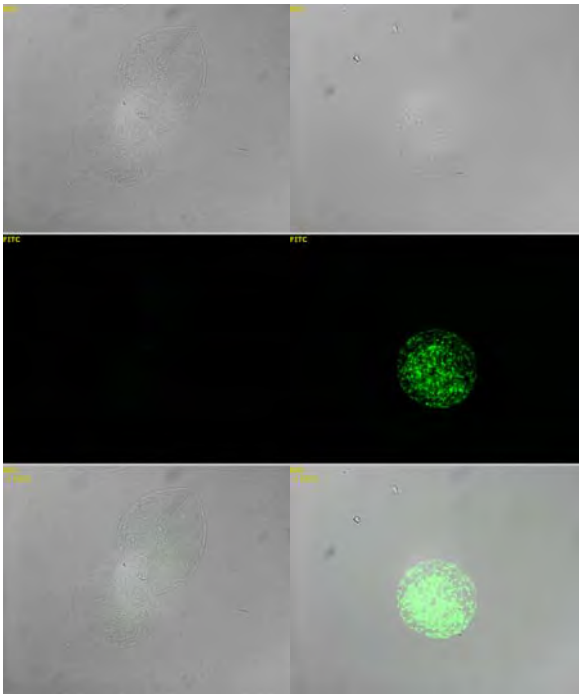

Figure 2. Representative images of Nile red fluorescence. The images on the left are negative controls (treated with absolute ethanol). The top images were obtained with differential interference contrast, the middle two are fluorescent images using a FITC filter and the bottom images are merged images.

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