Immunolocalization of CDX2 in Bovine Embryos – A Method for Differential Counting of Trophectoderm and Inner Cell Mass Cells

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Most techniques for determining numbers of trophectoderm (TE) and inner cell mass (ICM) cells utilize a permeabilization step in which TE cells are preferentially permeabilized and then stained with a nuclear dye. Excessive permeabilization can lead to an overestimate of numbers of TE cells and an underestimate of numbers of ICM cells. Conversely, inadequate permeabilization results in an underestimate of TE cells and an overestimate of ICM cells. For the current procedure, we take advantage of the fact that the transcription factor CDX2 is localized exclusively to TE to perform differential cell counting. Cells that are labeled with antiCDX2 and a nuclear dye (here DAPI but others can be used also) are considered TE whereas labeled nuclei that are negative for CDX2 are considered ICM.

NOTE: Perform all incubation steps in 96 well plates with 100 μl volume or, for primary antibody, 30-50 μl volume. An alternative is to perform some or all reactions in 25-50 μl microdrops under oil.

Materials and Reagents

- Triton X-100
- Tween 20
- Paraformaldehyde
- BSA (fraction V)
- PBS (one can use either Dulbecco's PBS for ease of preparation, 10 mM KPO_4, pH 7.4 containing 0.154 M NaCl or the same buffer with $NaPO_4$).
- Primary antibody – Anti-CDX2, (AM392-5M, Biogenex)
- Secondary antibody – FITC conjugated anti-mouse IgG (ab6785, Abcam) [it should be possible to substitute with a second antibody with different fluoroprobes such as AlexaFlour 555).
- D 4'-6-Diamidino-2-phenylindole (DAPI)
- Slides (Superfrost)
- Cover slips
- 96-well plates
- Antifade (Invitrogen)

Solutions

1. PBS/PVP – add 0.2 g polyvinyl pyrrolidone to 100 ml PBS; store at 4°C

2. PBST-BSA (1%) - add 0.10 ml Tween 20 and 1 g BSA to 100 ml PBS; store at 4°C

3. 4% Paraformaldehyde - 100 μL 8% (w/v) paraformaldehyde + 100 μL of PBS/PVP; prepare daily

4. Wash buffer - add 0.10 ml Tween 20 and 0.1 g BSA to 100 ml PBS; store at 4°C

5. Permeabilization solution – add 0.25 ml Triton X-100 to 100 ml PBS; store at 4°C

6. Blocking Buffer – add 5 g BSA to 100 ml PBS; store at 4°C)

7. Primary antibody (anti-CDX2) – sold ready-to-use.

8. Second antibody (anti-mouse IgG, FITC-labeled (1:1,000 in PBST-BSA)

9. Nuclear stain (DAPI) - prepare daily from 100x stock (100 µg/ml) – dilute 1:100 (v/v) in PBS/PVP.

Fixation

Fix embryos in 4% paraformaldehyde (solution 3) for 15 min at room temperature.

Wash the samples 3 times with ice cold PBS-PVP (solution 1). Use samples immediately or store for up to 1 week at 4°C.

Permeabilization

Incubate embryos for 20 min with permeabilization solution (solution 5) and wash embryos in wash buffer (solution 4 above).

Blocking and Incubation

Incubate embryos with blocking buffer (solution 6) for 1 h at room temperature.

Incubate cells in the ready-to-use CDX2 antibody overnight at 4°C.

Wash embryos three times in wash buffer (solution 4).

Incubate embryos with the FITC conjugated anti-mouse IgG (solution 8) for 1 h at room temperature in dark.

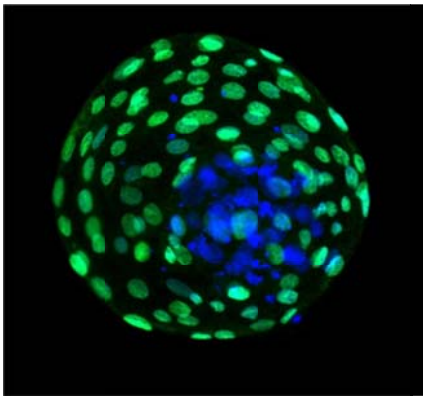
Wash embryos three times in wash buffer (solution 4).

Counterstaining

Incubate embryos with nuclear stain for 5 min.

Rinse with PBS-PVP.

Place embryos on a clean glass slide, affix a cover slip with Anti-fade solution, and seal with nail polish.



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