

Removal of Zona Pellucida with Pronase in Cattle

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Protease (Sigma-Aldrich P-8811), a proteolytic enzyme produced by *Saccharomyces griseus* and sometimes sold as Pronase®, can be used to remove the zona pellucida. Removal is important for a variety of purposes including for immunohistochemistry or to facilitate removal of all cumulus cells and thereby prevent contamination of protein or RNA extracts.

Materials

- 8% (w/v) paraformaldehyde stock solution: Dissolve 8 g of powdered paraformaldehyde in 100 ml water. Heat and stir (55-60 C – do not go higher). Add a few drops of 2 N sodium hydroxide until the solution clears. Make fresh each day. Alternatively, 8% paraformaldehyde can be purchased from [Electron Microscopy Sciences](#) as a custom formulation in 4 ml aliquots (cat. no. 15710-SP). Throw away whatever is not used in one day.
- 4% (v/v) paraformaldehyde: 1:1 solution of 8% paraformaldehyde stock solution and 0.2 M PBS. Make up on the day of use.
- Protease (0.1%, w/v) - weigh 0.1 g protease, add to a volumetric flask and add 100 ml of PBS (0.1 M). Aliquot in 1 ml tubes and store at -20 °C until use.

Protocol

- 1) Remove the embryos from the culture drop and wash them at least 3 times in PBS containing 1 mg/ml polyvinylpyrrolidone (PBS-PVP).
- 2) Put the embryos (as few as possible at each time) in a 50 µl drop of 0.1% protease and immediately watching them using a high magnification microscope that allows you to see the zona clearly. Leave the embryos in the drop until the zona is removed (up to 1 – 5 minutes). **BE CAREFUL!!!** Otherwise the protease will digest the embryo too!!!!!! Differences between stages of development from oocyte to expanded blastocyst may affect the time necessary for protease action.
- 3) Wash embryos very quickly 3 times in PBS-PVP and place them in a 50 µl drop of paraformaldehyde solution [4% (w/v) in PBS, pH 7.4]. The digestion seems to stop only when the embryos are in the paraformaldehyde drop (so be fast!!!).
- 4) Keep embryos in the paraformaldehyde drop for 1 h at room temperature for fixation.
- 5) Wash the embryos 3 times in a 50 µl drop PBS/ PVP by transferring the embryos from drop to drop (2 min for each wash).
- 6) Store the embryos at 4 °C in 4-well plates until the initiation of staining procedure.