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IMMUNOFLUORESCENCE PROTOCOL

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Designing protein-base...

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We use this protocol and it's working

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Abstract

This protocol describes the fixation and
immunofluorescent staining of mouse oocyte in paraformaldehyde solution.

Materials

1.6% formaldehyde, 100 mM PIPES (pH 7.0), 1 mM MgCl*2*, 0.1% Triton X-100, PBS, 3% BSA, primary antibodies, secondary antibodies, Hoechst 33342 (Invitrogen)

Protocol

1 Prepare fixation buffer consisting of 1.6% formaldehyde in 100 mM PIPES (pH 7.0), 1 mM MgCl2, and 0.1% Triton X-100.

2 Fix oocytes in fixation buffer at room temperature for 30 minutes.

3 Wash oocytes 4 times in PBS containing 0.1% Triton X-100 (PBT).

4 Incubate oocytes in PBT at 4 °C overnight.

5 Prepare blocking solution PBT containing 3% BSA.

6 Block oocytes in blocking solution at room temperature for 1 hour.

7 Prepare primary antibody solution in 3% BSA-PBT.

8 Incubate oocytes with primary antibodies at 4 °C overnight.

9 Wash oocytes 4 times in 3% BSA-PBT.

10 Prepare secondary antibody solution in 3% BSA-PBT containing 5 µg/ml Hoechst 33342 (Invitrogen).

11 Incubate oocytes in secondary antibody solution at room temperature for 2 hours or at 4 °C overnight.