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Protocol status: Working
We use this protocol and it's working

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Immunofluorescence Assay (IFA)

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ABSTRACT

Protocol for performing an immunofluorescence assay with fixed HeLa cells.

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Day 1

1 Wash HistoGrip (ThermoFisher) coated coverslips 1x in sterile PBS.

2 Seed cells in 6 well plate wells containing HistoGrip (ThermoFisher) coated coverslips in standard growth media, aiming for a confluency of ~70-80% at the time of treatment the next day.

Day 2

5h 35m

3 Feed cells for 01:00:00 prior to treatment with the desired growth compounds in standard growth media. 1h

4 At the conclusion of the treatment, aspirate all media from the well and replace with 500 µL /well of fixative ([M] 4 % (v/v) paraformaldehyde in [M] 0.1 Molarity (M) phosphate buffer). Incubate for 00:10:00 gently rocking at Room temperature . 10m

5 Remove the fixative, and replace with 500 µL /well of permeabilization buffer ([M] 0.1 % (v/v) Triton X-100 in PBS). Incubate for 00:10:00 gently rocking at Room temperature . 10m

6 Aspirate the permeabilization buffer, and replace with 500 µL /well of blocking buffer ([M] 3 % (v/v) goat serum in [M] 0.1 % (v/v) Triton X-100 in PBS). Incubate for 00:15:00 gently rocking at Room temperature . 15m

7 During step 4, make up the primary antibody solutions containing the desired antibodies in blocking buffer.

8 Aspirate the blocking buffer, and wash each well 3x with PBS.

9 Wash each well once with blocking buffer, and when aspirating the blocking buffer, gently nudge the coverslip into the center of the well.

10 Using a p200 pipette, carefully add 25 µL of the primary antibody solution directly onto the cover slip of each well. Ensure the bench this is performed on is flat. Replace the lid on each plate and cover all plates from light.

11 Incubate samples in the primary antibody solution for 02:00:00 at Room temperature (static, no rocking). During this incubation, make up the secondary antibody solutions containing the desired antibodies in blocking buffer. Cover this solution to protect it from light until it is needed. 2h

12 Wash each sample 3x in PBS.

13 Wash each well once with blocking buffer, and when aspirating the blocking buffer, gently nudge the coverslip into the center of the well.

14 Using a p200 pipette, carefully add 25 µL of the secondary antibody solution directly onto the cover slip of each well. Ensure the bench this is performed on is flat. Replace the lid of each plate and cover all plates from light.

15 Incubate samples in the secondary antibody solution for 02:00:00 at Room temperature (static, no rocking). 2h

16 Wash the samples 3x in PBS

17 Add PBS to each well to keep the coverslips hydrated prior to mounting.

18 Mount coverslips onto glass slides using a small amount of TRIS-buffered DABCO-glycerol mounting medium.

19 Seal the edges of each coverslip with black nail polish, and once dry, store slides protected from light at 4 °C until imaging.