PROTOCOL

IMMUNOFLUORESCENCE STAINING

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured in VitroGel system
- DPBS (Wash Buffer, no calcium, no magnesium)
- Fixation solution (4% formaldehyde solution)
- Permeabilization solution (0.1% Triton X-100)
- Blocking solution (3% BSA in DPBS)
- Primary and Secondary antibody
- Nucleus staining solution (eg: NucBlue™ Fixed Cell Stain ReadyProbes™ reagent from ThermoFisher Scientific # R37606)
- Micropipette; Low retention pipette tips
- Fluorescent microscope

PROTOCOLS: (using 96 well-plate, 50 µL gel/well as an example)

1. Remove the cover media from the top of the hydrogel.
2. Wash the hydrogel with DPBS: add 100 µL DPBS to the top of hydrogel and wait 1 minute before discarding. Wash 3 times.
3. Add 100 µL fixation solution and incubate at room temperature for 15-30 minutes.
4. Remove the fixation solution and wash 3 times with 100 µL DPBS.

   Optional stopping point: After removing Fixative, add 100 µL DPBS and parafilm the sample to prevent from drying out. Store at 4 °C for up to 1 month.

5. Add 100 µL permeabilization solution and incubate at room temperature for 5 minutes.

   Note: Permeabilization solution may cause the hydrogel to soften or slowly dissolve if incubate more than 15 min. Incubate at 4 °C if more than 5 minutes.

6. Remove the permeabilization solution and carefully wash 3 times with 100 µL DPBS.
7. Add blocking solution and incubate 30-60 minutes at room temperature.
8. Add desired primary antibody directly to the blocking solution covering the hydrogel to yield a 1X final dilution, mix gently, and incubate overnight at 4 °C.
9. Remove the solution with primary antibody and carefully wash 3 times with 100 µL DPBS.
10. Add the appropriate secondary antibody (diluted to 1X in blocking solution) and incubate in the dark for 3-6 hours at room temperature or 4 °C.
11. Remove the solution with the secondary antibody and carefully wash 3 times with 100 µL DPBS.
12. Add nucleus staining solution* and incubate in the dark for 5 minutes at room temperature. After incubation, the sample is ready for fluorescent imaging.

    *Prepare the working solution of nucleus staining solution according to the product manual: add 2 drops of the reagent to 1 mL of DPBS.

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IMPORTANT NOTES:

- This protocol is suitable for both 3D hydrogel culture and 2D hydrogel coating culture.
- If different staining solutions are used, prepare the staining solution according to the product manual. The final concentration of staining solution and incubation time might need to be optimized depending on the different cell types and hydrogel sizes.
- During the washing steps, carefully add or remove the solution to avoid possible loss of hydrogel/cells.