PROTOCOL

CELL FLUORESCENT STAINING IN GEL FOR NUCLEI AND ACTIN FILAMENT

RECOMMENDED MATERIALS AND REAGENTS

- Cells cultured with VitroGel system
- DPBS (Wash Buffer, no calcium, no magnesium)
- Micropipette
- Fixation solution (4% formaldehyde solution)
- Permeabilization solution (0.1% Triton X-100)
- Blocking solution (3% BSA in DPBS)
- F-actin filament staining solution (eg: ActinGreen™ 488 ReadyProbes™ reagent from ThermoFisher Scientific # R37110)
- 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.
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 incubated 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 100 µL blocking solution and incubate for 60 minutes at room temperature.
8. Remove the blocking solution, add 100 µL F-actin staining solution*, and incubate in the dark for 30-60 minutes at room temperature.
9. Remove the F-actin staining solution and wash 3 times with DPBS.
10. 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 F-actin staining solution and nucleus staining solution according to the product manual: add 2 drops of the reagent to 1 mL of DPBS.

PROTOCOL

CELL LYSIS FOR DOWNSTREAM ANALYSIS

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.

Corporate Headquarters
TheWell Bioscience Inc.
675 US Highway 1
North Brunswick, NJ 08902 US TF: +1 866.3D.CELLS TEL: +1 973.855.4955 | support@thewellbio.com | www.thewellbio.com