RASTRUM

Protocol

In Situ Immunofluorescence Analysis

Document Number | WET.BCM.0008.PRO.v1.02
Date of issue | September 2020

INVENTIA
INSPIRING SCIENCE

Introduction

This protocol outlines a method to analyse protein expression via immunofluorescence in 3D cell models created with RASTRUM. This protocol takes three (3) days and is designed for 3D cell models printed into a 96 well plate.

Equipment and reagents required but not provided

- 4% Paraformaldehyde (PFA)
- Blocking solution
- Phosphate-buffered saline (PBS)
- PBST (0.1% v/v Tween-20 in PBS)
- 0.1% Triton-X-100 in PBS
- Primary (1°) and secondary (2°) antibodies
- Hoechst 33342 (Thermo, cat #H3570)
- Fluorescence microscope

Protocol

Day 1

1. Remove and discard culture medium and add 150 µL PBS to each well.
2. Remove PBS and discard and add 100 µL 4% PFA to each well
3. Incubate plate at room temperature, 20 minutes.
4. Remove and discard 4% PFA and wash each well with 150 µL PBS, three times.
5. Remove and discard PBS and add 100 µL 0.1% Triton-X-100 to each well.
6. Incubate plate at room temperature, 30 minutes.
7. Remove and discard Triton-X-100 and wash each well with 150 µL PBS, 10 minutes, three times.

Note: All waste from Steps 5-8 should be disposed of according to institution-specific laboratory waste disposal guidelines.

8. Remove PBS and add 100 µL blocking solution to each well to block non-specific antibody binding.
9. Incubate plate at room temperature, 30 minutes.
10. Prepare 1° antibody working solution in blocking solution.
11. Remove and discard blocking solution and add 100 µL primary antibody working solution to each well.
12. Cover plate with aluminium foil and incubate at 4°C, overnight.

Day 2

13. Remove and discard 1° antibody and wash each well with 150 µL PBST, 10 minutes, three times.
14. Prepare 2° antibody working solution in blocking solution.
15. Remove and discard PBST and add 100 µL 2° antibody working solution.
16. Cover plate with aluminium foil and incubate at 4°C, overnight.

Day 3

17. Remove and discard 2° antibody and wash each well with 150 µL PBST, 10 minutes, three times.
18. Prepare 5 µg / mL Hoechst 33342 working solution in PBS.
19. Remove and discard PBST and add 100 µL Hoechst 33342 working solution.
20. Incubate plate at room temperature, 10 minutes.
21. Remove and discard Hoechst 33342 working solution and add 150 µL PBS to each well.
22. Remove and discard PBS wash, and add 150 µL PBS to each well.
23. Proceed to imaging with a fluorescence microscope.

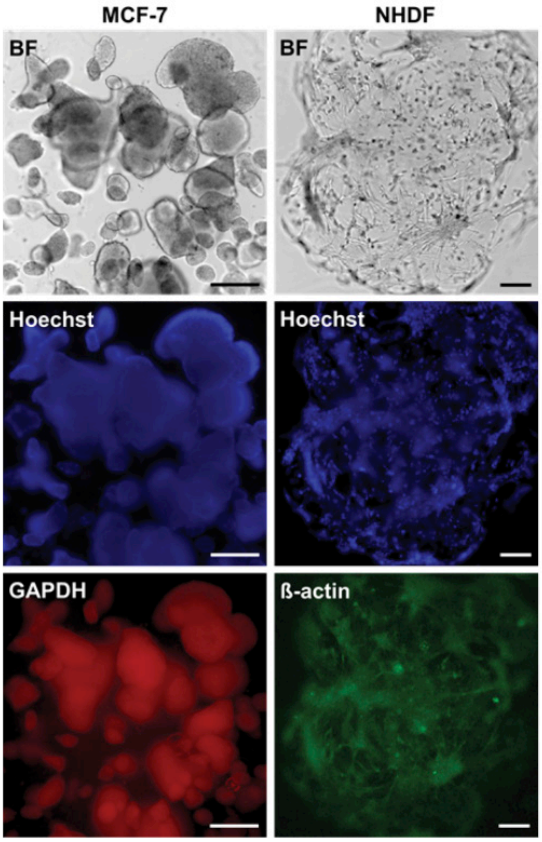

Figure 1: Representative brightfield (BF), Hoechst and GAPDH/β-actin immunofluorescent images of MCF-7 breast cancer and Neonatal human dermal fibroblast (NHDF) cells encapsulated in RASTRUM hydrogel matrices 7 days post-printing. Scale bars = 200 µm

INVENTIA

Inventia Life Science Operations Pty Ltd
ABN 19 613 078 710
Suite 1.13, 90-96 Bourke Road,
Alexandria, NSW, 2015, Australia
Telephone +61 1800 849 128
info@inventia.life | www.inventia.life