3D Gel Staining Prep Kit

(3D-GSP)
Cat #8808

Product Description

The 3D Gel Staining Prep Kit (3D-GSP) is designed to be a companion product for ScienCell's collagen I-based 3D culturing kits. This kit includes reagents necessary to fix resultant gels and their embedded cells, permeabilize the gel, and incubating buffers to stain the gels with antibodies of your choice. The reagents and protocol included in this kit are optimized for immunofluorescence staining of cells in collagen-based 3D gels.

Kit Components

Cat #	# of vials	Name	Quantity	Storage
8808-a	1	4% Paraformaldehyde (PFA)	20 mL	-20°C
8808-b	1	Quenching Buffer	20 mL	2-8 °C
8808-c	1	Permeabilization Buffer	20 mL	2-8 °C
8808-d	1	Blocking Buffer	20 mL	2-8 °C
8808-e	1	Antibody Incubation Buffer	20 mL	2-8 °C

Not Included: Additional Recommended Materials

Cat #	Product Name
0303	Dulbecco's Phosphate-Buffered Saline (DPBS)
-	VECTOR Laboratories VECTASHIELD Mounting Medium, Cat #H-1200

[Note: Kit does not include antibodies or mounting solution. Required antibody concentrations and antibody incubation times may differ according to your specific antibodies.]

Quality Control

3D-GSP is ensured to fix and permeabilize 3D gels resultant from ScienCell's 3D collagen I-based culturing kits for immunofluorescence staining when used according to the included protocol. Not a sterile product.

Product Use

3D-GSP is for research use only. It is not approved for human or animal use, or application in clinical or *in vitro* diagnostic procedures.

Shipping

8808-a is shipped on dry ice; all other components are shipped on gel ice.

Procedure:

Note before starting: This kit is designed to be a companion product to ScienCell's collagen I-based 3D culturing kits #8708, 8728, and 8698. The following protocol assumes Cat. #8708, 8728, and 8698 were used according to their included protocols. This kit is designed to stain gels adhered to a 24-well plate using immunofluorescence.

Note: Please see accompanying SDS for precautions and approved waste disposal for paraformaldehyde.

1. Thaw 4% paraformaldehyde (PFA, #8808-a) either overnight at 4 °C or for a few hours at room temperature. Do not thaw PFA in a water bath.
2. Carefully remove media from adhered collagen-I gels in their 24-well plate and wash three times with 1x DPBS.
3. Add 350 µL of 4% PFA to each well of the 24-well plate containing a gel intended for staining. Incubate for 20 minutes with rocking at room temperature. Exercise caution when handling 4% PFA.
4. Discard 4% PFA into an appropriate collection receptacle for proper waste disposal.
5. Add 350 µL Quenching Buffer (#8808-b) to each fixed gel and incubate for 15 minutes with rocking at room temperature.
6. Remove the 150 mM glycine from each fixed gel.
7. Wash gels for 10 minutes twice with DPBS.
8. Add 500 uL of permeabilization buffer (8678-c) to each fixed gel and incubate 20 minutes at room temperature with rocking.
9. Remove the permeabilization buffer from each fixed gel.
10. Add 500 µL of blocking buffer (8678-d) to each fixed gel and incubate overnight at 4 °C or for 1 hour at room temperature with rocking.
11. Remove the blocking buffer from each fixed gel. Fixed gels can be stored at this point in 1X DPBS at 4 °C for at least 1 week.
12. When ready for staining, carefully dislodge the fixed gel in DPBS from the 24-well plate using a standard 200 µL pipette tip. Use the pipette tip to lift the edges of the gel first and carefully continue to dislodge the gel until it is no longer attached.
13. Optional: use forceps to remove the gel from the DPBS and use a scalpel to cut the gel into halves or quarters on a microscope slide. Halves or quarters may then be stained separately.
14. Use forceps to move the fixed gels into a wells of a 48-well plate.
15. Incubate fixed gels in the 48-well plate using your primary antibodies of choice diluted appropriately in antibody incubation buffer (#8808-e) for 1 hour at room temperature or overnight at 4 °C with rocking. *Note*: antibody incubation times and dilutions may vary depending on the antibody. Test your antibody quality prior to use with 3D gel staining.
16. Wash gels three times for 10 minutes each with DPBS. Do not use an aspirator to remove incubating solution or DPBS or gels may be lost.
17. Incubate fixed gels with your secondary antibodies of choice diluted appropriately in antibody incubation buffer (8808-e) for 1 hr at room temperature with rocking. Note: antibody incubation times and dilutions will vary depending on the antibody. A secondary antibody dilution of 1:1000 is usually sufficient for 30 minutes to 1 hour.
18. Wash gels three times for 10 minutes each with DPBS. Do not use an aspirator to remove incubating solution or DPBS or gels may be lost.
19. Mount fixed gels using an appropriate mounting medium and an appropriately sized coverslip onto a microscope slide. We recommend VectaShield for IF.
20. Sealed slides in VectaShield can be viewed for at least 1 week.