

PURAMATRIX™ IMMUNOSTAINING PROTOCOL



IMMUNOSTAINING-PRIMARY AND SECONDARY ANTIBODY PROTOCOL

For immunostaining, there are two major concerns. First is the need for the antibodies to diffuse readily through the PuraMatrix nanofibrous peptide scaffolds to reach the cells. Second, is the need to block any non-specific binding of the antibodies to PuraMatrix. The protocol below is written for cells that have been grown in 3D in inserts (please refer to the document *PuraMatrix Insert 3-D Plating Protocol* but it can also be used on cells that have been plated in wells (either 3D or 2-D surface-plated).

- 1) Gently remove media from culture well.
- 2) Fix cells in gels in 4% paraformaldehyde for 30 minutes.
- 4) Wash in PBS.
- 5) Block in Block Solution (PBS + 10% Fetal Bovine Serum (FBS)).
 - a) Block solution should be changed every few hours and left over night.
- 6) Add primary antibody in block solution. Leave overnight at 4°C.
 - a) We recommend that a higher concentration of primary antibody be used when cells are staining in PuraMatrix than used for just cells alone.
 - b) We highly recommend a “no-primary” control is needed (do not add primary antibody to one sample, then add the secondary to assess background of secondary alone).
 - c) Make sure to use a large enough volume to soak the gel in the insert.
- 7) Wash in block solution at least 4 times for a few hours each.
- 8) Add secondary antibody in block solution.
 - a) Again, we recommend a higher concentration of antibody than used for cells alone.
 - b) Leave for 4 hours.
- 9) Wash again in PBS or block solution multiple times (4-6X) for at least an hour, carefully remove gel from insert, place on glass slide and view under microscope.

By increasing the concentration of antibodies and wash steps, one should achieve a strong and specific signal.

MORPHOLOGICAL ANALYSIS: For morphological analysis, using a fluorescent phalloidin (overnight in PBS at the recommended concentration) nicely stains the actin cytoskeleton of all cells and can be combined with DAPI to view nuclei (see page 2).

IMMUNOSTAINING-RHODAMINE-PHALLOIDIN PROTOCOL

- 1) Gently remove media from culture well.
- 2) Add 4% paraformaldehyde (PFA) fixative for 20 minutes.
- 3) Gently remove.
- 4) Add desired stain.
 - a) We recommend (160 nM) rhodamine-phalloidin in PBS (25 ul/ml soln from Molecular Probes), 300 ul/well into inserts.
 - b) Cover and leave overnight.
 - c) Add 1X DAPI for 1 hour while taking volume to 1 ml w/ PBS.
- 5) Change PBS buffer by removing the 1 ml PBS and add another 1 ml PBS.
- 6) Remove insert from well.
- 7) Next, remove PBS from the top of the insert and carefully remove gel from insert, place on glass slide and view under microscope.

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