

Immunostaining Protocol for formalin fixed paraffin-embedded section for anti-mouse Podoplanin

1. Deparaffinize through xylenes, ethanol , and hydrate to water.
2. 1% hydrogen peroxide in methanol , 30-40 min., room temp
3. Wash 2x, dd water, wash in Tris buffer, pH 7.2, 5 min. each.
4. Proteinase K in 0.2M Tris, buffer, pH 7.2, 37 degrees C, 36 ug/ml, 30 min.
5. Wash 3x, 5 min each: Tris buffer, pH 7.2, dd sterile water; TNT wash buffer.
6. Block in TNB blocking buffer, 30 min. RT (according to instructions of NEN TSA-Indirect kit, cat. # NEL 700A).
7. Primary antibody: hamster anti-mouse Podoplanin antibody 5-10 µg/ml in TNB buffer, overnight, 4 degree.
8. Rinse in TNT wash buffer, 3x, 5 min
9. Incubate with rabbit anti-syrian hamster IgG HRP conjugated, 1:100 in TNB buffer, 30 min. RT. Wash 3x.
10. Biotinylated-tyramide in amplification diluent, 1:50 , 7 minutes, RT Wash 3x.
11. Incubate in SA-HRP , 1:100 in TNB, 30 min, RT. Wash 3x.
12. DAB
13. Counter stain.