

Immunohistology with cryosections using TIE-2 antibodies clone 2, 9 and 16

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Cryosections of human organs were prepared on silanized slides, allowed to dry and stored at -20°C overnight. The sections were immediately fixed in the frozen state using isopropanol at 4°C for 10 min. carefully avoiding any condensation or thawing before fixation. During the entire staining procedure the sections were never allowed to dry. Endogenous peroxidase was first inactivated using a glucose oxidase procedure (see below). Mabs TIE-2 clone 9 and clone 16 were used at a dilution of 1:300 in PBS containing 1% BSA and 0.1% NaN₃, TIE-2 mAb clone 2 was used at 1:50 to 1:80. After washing the fixed sections in PBS and carefully removing excess fluid by a standardized procedure, 20µl of antibody solution were applied per section at 4°C in a moist chamber overnight. After washing again, the Vectastain ABC Elite Kit for mouse IgG and peroxidase (Vector Laboratories, Burlingame, USA, No. BA-9200) was then applied according to the manufacturer's recommendations. The biotinylated anti-mouse IgG in this kit is absorbed against human immunoglobulins and thus normal human serum was not needed in the secondary antibody solution.

Finally, peroxidase activity was revealed using the standard diaminobenzidine reaction. The sections were then dehydrated in a graded series of isopropanol concentrations applying finally two xylene baths and Eukitt as mounting medium for coverslipping.

The antibodies do not work with formalin-fixed paraffin sections!

Glucose oxidase procedure for inactivation of endogenous peroxidase in cryosections:

Final concentration of reagents:

10 mM glucose (Merck No. 8342)

1 mM sodium azide (Baker No. 97167)

0.4 U/ml glucose oxidase (Sigma No. G-6641)

in PBS, pH 7.2

This procedure is mandatory if, for example, lymphatic organs are stained. Pre-heat the solution to 37°C and apply it to pre-heated sections for 45 min at 37°C in a Coplin jar and a water bath carefully monitoring the temperature close to the sections. The procedure leads to high concentrations of H₂O₂ only in the immediate vicinity of peroxidase-expressing cells. Always pay attention to the specific activity of glucose oxidase mentioned in the data sheet, because this may vary with different shipments from the same supplier!