TB4 Fluorescence Immunohistochemistry

Forked from Fluoresence immunohistochemistry

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ABSTRACT

Protocol describing fluorescence immunohistochemistry for detection of thymosin beta 4 alongside CD31, smooth muscle actin and myosin heavy chain in PFA-fixed human cardiac tissue samples.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Saunders V, Dewing JM, Sanchez-Elsner T, Wilson DI (2018) Expression and localisation of thymosin beta-4 in the developing human early fetal heart. PLoS ONE 13(11): e0207248. doi: 10.1371/journal.pone.0207248

PROTOCOL STATUS

Working

1 Tissue was fixed overnight in 4% PFA and then dehydrated the following day with 2 hour washes of 70%, 80%, 90% and 100% ethanol. Tissue was then placed in 100% chloroform overnight. The following day the tissue was embedded in paraffin wax, allowed to set and then stored at 4ºC.

2 Using a Leica rotary microtome, 10 micron thick sections were cut from tissue blocks and mounted onto aminoalkylsilane-coated glass slides. These sections were stored at 4ºC.

3 Prior to staining, sections were de-paraffinised and rehydrated with washes of xylene, ethanol (100%, 90% and 70%) and distilled water. Antigen retrieval was then carried out by boiling slides in 0.01 mol/L sodium citrate solution (pH 6.0) + 0.05% Tween 20 for 15 mins.

4 Sections were stained with the appropriate primary and secondary antibodies. All antibodies were diluted in PBS + 0.1% Triton X-100 + 3% serum. Primary antibodies were typically incubated overnight at 4ºC while secondary antibodies were incubated for 1 hour at room temperature. For triple-stained sections, the secondary antibody treatments were carried out consecutively, if required, to prevent cross-reactivity. Sections stained with the appropriate secondary antibodies alone were used as negative controls.

5 The primary antibodies used were as follows:
rabbit anti-thymosin β4 polyclonal antibody used at 1:1500 dilution (Millipore Cat# AB6019, RRID:AB_10806893); mouse anti-myosin heavy chain monoclonal antibody used at 1:50 dilution (Thermo Fisher Scientific Cat# 50-6503-80, RRID:AB_2574266); mouse anti-SMA monoclonal antibody used at 1:100 dilution (Leica Microsystems Cat# NCL-SMA, RRID:AB_442134); sheep anti-CD31 polyclonal antibody used at 1:2000 dilution (R and D Systems Cat# AF806, RRID:AB_355617).

The secondary antibodies used were as follows:
goat anti-rabbit IgG-FITC at 1:200 dilution (Sigma-Aldrich Cat# F6005, RRID:AB_259682); goat anti-mouse IgG-Alexa Fluor 594 at 1:200 dilution (Thermo Fisher Scientific Cat# A-11005, RRID:AB_2534073); donkey anti-sheep IgG-Alexa Fluor 555 at 1:400 dilution (Thermo Fisher Scientific Cat# A21436, RRID:AB_10376163).

6 Following staining, sections were dehydrated by 2 min treatments in 70%, 90% and then 100% ethanol followed by two 2 min treatments in xylene.

7 Sections were placed in 100% ethanol before being mounted and the nuclei counterstained using Vectashield Mounting Medium with DAPI (Vector Laboratories) and the slides sealed with a coverslip. The stained slides were stored at 4°C.

8 Slides were imaged using a standard fluorescence microscope or a Leica SP5 confocal microscope.