

immunofluorescence analysis of myosin heavy chain isoforms

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Abstract

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Materials

BA-F8 (MHC I) BA-F8 by [Developmental Studies Hybridoma Bank](#)
SC-71 (MHC IIa) SC-71 by [Developmental Studies Hybridoma Bank](#)
BF-F3 (MHC IIb) BF-F3 by [Developmental Studies Hybridoma Bank](#)
AlexaFluor 350 Goat anti-mouse IgG2b A-21140 by [Gibco - Thermo Fischer](#)
AlexaFluor 488 Goat anti-mouse IgG1 A-21121 by [Gibco - Thermo Fischer](#)
AlexaFluor 568 Goat anti-mouse IgGM A-21043 by [Gibco - Thermo Fischer](#)

Protocol

Step 1.

Cut frozen muscle sections (5 mm) in a cryostat on microscope slides.

Step 2.

The slides were allowed to reach room temperature.

Step 3.

The slides were permeabilized with 0.3% Triton X-100-PBS for 10 min at 4°C.

Step 4.

A blocking solution of 5% normal goat serum (NGS)-PBS was applied for 1 h at room temperature.

Step 5.

Primary antibodies in 1% NGS-PBS (1:100) were applied at 4°C overnight.

Step 6.

Three consecutive washes with PBS for 5 min each.

Step 7.

Secondary antibodies in 1% NGS-PBS (1:500) were applied for 30 min at room temperature.