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MyHC-IIb/Laminin Fresh Frozen

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Griffen Wakelin¹

¹Dalhousie University



Griffen Wakelin

Dalhousie University

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Protocol status: Working

We use this protocol and it's working

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Abstract


Myosin heavy chain IIb/laminin immunofluorescence staining protocol for fresh frozen skeletal muscle.

Protocol materials


 M.O.M.® (Mouse on Mouse) Blocking Reagent **Vector Laboratories Catalog #MKB-2213-1** Step 2

 BF-F3 (MHC IIb) **Developmental Studies Hybridoma Bank Catalog #BF-F3** Step 6

 Anti-Laminin antibody produced in rabbit **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L9393** Step 6

 Alexa Fluor® 647 AffiniPure™ Goat Anti-Mouse IgM, μ chain specific **Jackson ImmunoResearch Laboratories, Inc. Catalog #115-605-075**

Step 10

 Alexa Fluor® 488 AffiniPure™ Goat Anti-Rabbit IgG (H L) **Jackson ImmunoResearch Laboratories, Inc. Catalog #111-545-003**

Step 10

 DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9542** Step 10



Staining

3h 45m

- 1 Thaw sections, dry for ~30 mins, pap, and label.

Note

The MyHC-IIb will, under no circumstances, work with fixed tissue. Heat-induced anti retrieval has been reported to work:

CITATION

Esper ME, Kodippili K, Rudnicki MA. (2022). Immunofluorescence Labeling of Skeletal Muscle in Development, Regeneration, and Disease. *Methods in Molecular Biology*.

LINK

https://doi.org/10.1007/978-1-0716-2675-7_9

however, we have not been able to get this to work ourselves.

- 2 Block

1h



M.O.M.® (Mouse on Mouse) Blocking Reagent **Vector Laboratories Catalog #MKB-2213-1**



01:00:00



Room temperature

- 3 Wash 1X PBS



00:05:00

5m

- 4 Wash 1X PBS



00:05:00

5m

- 5 Wash 1X PBS



00:05:00

5m

- 6 Incubate primaries in 1% BSA:

- BF-F3 (MHC IIb) **Developmental Studies Hybridoma Bank Catalog #BF-F3** 1:10

- Anti-Laminin antibody produced in rabbit **Merck MilliporeSigma (Sigma-Aldrich) Catalog #L9393**

1:1000














Overnight



4 °C



- 7 Wash 1X PBS  00:05:00 5m
- 8 Wash 1X PBS  00:05:00 5m
- 9 Wash 1X PBS  00:05:00 5m
- 10 Incubate secondaries in 1% BSA: 2h
 -  Alexa Fluor® 647 AffiniPure™ Goat Anti-Mouse IgM, μ chain specific **Jackson ImmunoResearch Laboratories, Inc. Catalog #115-605-075**
1:1000
 -  Alexa Fluor® 488 AffiniPure™ Goat Anti-Rabbit IgG (H L) **Jackson ImmunoResearch Laboratories, Inc. Catalog #111-545-003**
1:1000
 -  DAPI **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D9542** 1 μ g/ml

 02:00:00  Room temperature
- 11 Wash 1X PBS  00:05:00 5m
- 12 Wash 1X PBS  00:05:00 5m
- 13 Wash 1X PBS  00:05:00 5m
- 14 Mount with aqueous mounting media. Image within 2 days.

Citations

Step 1

Esper ME, Kodippili K, Rudnicki MA.. Immunofluorescence Labeling of Skeletal Muscle in Development, Regeneration, and Disease

https://doi.org/10.1007/978-1-0716-2675-7_9