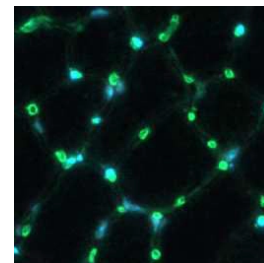


Jun 05, 2024 Version 2

## Twist2 Snap Frozen Skeletal Muscle IF V.2

This protocol is a draft, published without a DOI.



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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol details Twist2 immunofluorescent staining of fresh frozen mouse skeletal muscle.

## Image Attribution

Representative Twist2 stain in skeletal muscle. Griffen Wakelin. June 1st, 2024. Dalhousie University.



## Protocol materials

⊗ Fluoromount **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F4680** Step 11

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

Step 9

⊗ DAPI **Invitrogen - Thermo Fisher Catalog #D3571** Step 9

⊗ Anti-Twist2 antibody **Abcam Catalog #ab66031** Step 7

⊗ Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7030** Step 6

⊗ Triton X-100 **Fisher Scientific Catalog #BP151-100** Step 4

⊗ Paraformaldehyde **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6148** Step 2

⊗ Hydrophobic Barrier Pen **Vector Laboratories Catalog #H-4000** Step 1

## Before start

Collect fresh muscle according to institution-specific guidelines and snap freeze in liquid nitrogen-cooled 2-methylbutane. The Twist2 stain does not work on PFA-fixed muscle without antigen retrieval.



## Day 1

10m


- 1 Thaw slides, dry for 30 mins, surround sections with  
 Hydrophobic Barrier Pen **Vector Laboratories Catalog #H-4000** , and label
- 2 Fix with ice-cold [M] 4 % (v/v) PFA/PBS  
 Paraformaldehyde **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6148** for  
⌚ 00:05:00
- 3 Wash three times with 1X PBS for ⌚ 00:05:00
- 4 Permeabilize with [M] 0.2 % (v/v) Triton/PBS  
 Triton X-100 **Fisher Scientific Catalog #BP151-100** for ⌚ 00:10:00
- 5 Wash three times with 1X PBS for ⌚ 00:05:00
- 6 Block with [M] 5 Mass / % volume BSA/PBS  
 Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich) Catalog #A7030** for  
⌚ 01:00:00 at 🌡 Room temperature
- 7 Incubate primary Anti-Twist2 antibody **Abcam Catalog #ab66031** 1:500 in 1% BSA  
⌚ Overnight at 🌡 4 °C

## Day 2

30m

- 8 Wash three times with 1X PBS for ⌚ 00:05:00
- 9 Incubate secondary  
 Alexa Fluor® 488 AffiniPure™ Goat Anti-Rabbit IgG (H+L) **Jackson ImmunoResearch Laboratories, Inc. Catalog #111-545-003**  
1:1000 and DAPI **Invitrogen - Thermo Fisher Catalog #D3571** 1:1000 in 1% BSA for  
⌚ 02:00:00 at 🌡 Room temperature




10 Wash three times with 1X PBS for  00:05:00

5m



11 Mount with aqueous mounting media such as

 Fluoromount **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F4680** . Image within 2 days.