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## Twist2 Fresh Frozen

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**Protocol status:** In development

**We are still developing and  
optimizing this protocol**

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## Abstract

Immunofluorescence protocol for Twist2 staining of fresh frozen mouse tissue.

## Protocol materials

 Anti-Twist2 antibody **Abcam Catalog #ab66031** Step 12

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

Step 16







## Stain




10m

- 1 Thaw slides, dry for 30 mins. Pap and label.
- 2 Post-fix with ice-cold PFA [M] 4 % (v/v) PFA/PBS ⌚ 00:05:00 5m
- 3 Wash with 1X PBS ⌚ 00:05:00 5m
- 4 Wash with 1X PBS ⌚ 00:05:00 5m
- 5 Wash with 1X PBS ⌚ 00:05:00 5m
- 6 Permeabilize with Triton X-100 [M] 0.2 % (v/v) Triton/PBS ⌚ 00:10:00 10m
- 7 Wash with 1X PBS ⌚ 00:05:00 5m
- 8 Wash with 1X PBS ⌚ 00:05:00 5m
- 9 Block with 5% BSA ⌚ 01:00:00 🌡 Room temperature 1h
- 10 Wash with 1X PBS ⌚ 00:03:00 3m
- 11 Wash with 1X PBS ⌚ 00:03:00 3m
- 12 Incubate primary Anti-Twist2 antibody **Abcam Catalog #ab66031** 1:500 in 1% BSA  
⌚ Overnight 🌡 4 °C



- 13 Wash with 1X PBS  00:05:00 5m
- 14 Wash with 1X PBS  00:05:00 5m
- 15 Wash with 1X PBS  00:05:00 5m
- 16 Incubate secondary  

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

  
1:1000 and DAPI 1:1000 in 1% BSA.
- 17 Wash with 1X PBS  00:05:00 5m
- 18 Wash with 1X PBS  00:05:00 5m
- 19 Wash with 1X PBS  00:05:00 5m
- 20 Mount with aqueous mounting media. Image within 2 days.