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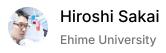
# Immunofluorescence protocol of Pax7 and androgen receptor for frozen muscle sections with unmasking

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### Abstract

Immunofluorescence protocol of Pax7 on muscle tissues is a critical step for studying skeletal muscle regeneration. Here, I describe the simple protocol for Pax7 for isopentane-frozen muscle tissues with unmasking. Androgen receptor, a member of the nuclear receptor superfamily, can be stained with this protocol to see the double positive cells.

## Guidelines

Use **freshly cut sections** since Pax7 and AR on sections are not stable.

#### **Materials**

#### **MATERIALS**

- Anti-Androgen Receptor antibody [SP107] N-terminal Abcam Catalog #ab105225
- Goat anti-Rabbit IgG (H L) Cross-Adsorbed Secondary Antibody Alexa Fluor 488 Thermo Fisher Scientific Catalog #A-11008
- ProLong™ Glass Antifade Mountant Invitrogen Thermo Fisher Catalog #P36980
- Anti-Pax7 antibody Developmental Studies Hybridoma Bank Catalog #pax7
- Goat anti-Mouse IgG1 Cross-Adsorbed Secondary Antibody Alexa Fluor 568 Thermo Fisher Scientific Catalog #A-21124

## **Troubleshooting**

## Before start

#### **REAGENT SETUP**

## Sodium citrate (10 mM, pH = 6)

- A solution (Keep at room temperature)
  - 2.1 g of Citric Acid Monohydrate (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> · H<sub>2</sub>O)
  - 100 ml of H<sub>2</sub>O
- -B solution (Keep at room temperature)
  - 14.7 g of Trisodium citrate dihydrate (C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub> · 2H<sub>2</sub>O)
  - 500 ml of H<sub>2</sub>O
- Sodium citrate
  - 9 ml of A solution
  - 41 ml of B solution
  - 450 ml of H<sub>2</sub>O



## Fix

1 Fix the slides with 4% PFA/PBS for 5 min at room temperature.

#### Note

Do not air dry the samples before fixing. It may increase the background signal and lose androgen receptoer signal.

2 Wash the slides in PBS three times for 5 min.

## **Unmasking**

- 3 Place the slides into a slide chamber in PBS
- 4 Fill a beaker with 500 ml of Sodium citrate.
- 5 Place the beaker on the hotplate and turn it on full power.

### Note

While wating for the beaker to come to a boil, keep the sections in PBS.

6 Once boiling, transfer the slides from PBS to the beaker.

#### Note

Use care with hot solution. Use forceps.

- 7 Boil the slides for 10 min.
- 8 Remove the beaker from the hotplate and let the beaker cool for 20 min.
- 9 Remove the slide chamber from the beaker.



Wash slides in PBS three times for 5 min.

# **Blocking**

- Block the slides with 5% Goat serum/0.05% Triton X-100/PBS for 1h at room temperature. Prepare 100 μl of 5% Goat serum/0.05% Triton X-100/PBS per slide.
- Drain the slides for a few seconds (do not rinse) and wipe around the sections with tissue paper.

## **Primary antibodies**

- Dilute primary antibodies as follows in 5% Goat serum/0.05% Triton X-100/PBS. Prepare 100 μl antibody solution per slide. Add primary antibody solution to the slides.
  - △ 100 μL 5% Goat serum/0.05% Triton X-100/PBS
  - $\triangle$  5 µL anti-Pax7 (mouse IgG1, 1/20)
  - △ 0.25 μL anti-androgen receptor (rabbit, 1/400)
- 14 Incubate overnight at 4°C.
- Wash the slides in PBS three times for 5 min.

# Secondary antibodies

- Dilute secondary antibodies as follows in 5% Goat serum/0.05% Triton X-100/PBS.

  Prepare 100 μl antibody solution per slide. Add secondary antibody solution to the slides.
  - △ 100 μL 5% Goat serum/0.05% Triton X-100/PBS
  - $\perp$  0.1 µL anti-mouse IgG1 568 (1/1000)
  - $\perp$  0.1  $\mu$ L anti-rabbit IgG 488 (1/1000)
  - $\Delta$  0.05  $\mu$ L DAPI (1/2000)
- 17 Incubate for 1h at room temperature protected from light.



18 Wash the slides in PBS three times for 5 min.

# Mount

Mount using  $\operatorname{ProLong}^{\operatorname{m}}$  Glass Antifade Mountant and add coverslips. 19