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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 ($C_6H_8O_7 \cdot H_2O$)
  - 100 ml of H_2O
- B solution (Keep at room temperature)
  - 14.7 g of Trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$)
  - 500 ml of H_2O
- Sodium citrate
  - 9 ml of A solution
  - 41 ml of B solution
  - 450 ml of H_2O

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.

10 Wash slides in PBS three times for 5 min.

Blocking

11 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.

12 Drain the slides for a few seconds (do not rinse) and wipe around the sections with tissue paper.

Primary antibodies

13 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
- 5 µL anti-Pax7 (mouse IgG1, 1/20)
- 0.25 µL anti-androgen receptor (rabbit, 1/400)

14 Incubate overnight at 4°C.

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

Secondary antibodies

16 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
- 0.1 µL anti-mouse IgG1 568 (1/1000)
- 0.1 µL anti-rabbit IgG 488 (1/1000)
- 0.05 µL 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

19 Mount using ProLong™ Glass Antifade Mountant and add coverslips.