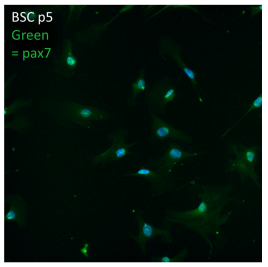
Feb 12, 2019 Version 1

Bovine satellite cell Pax7 ICC V.1



Forked from Bovine satellite cell Pax7 ICC

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Protocol status: Working
We use this protocol and it's working

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Abstract

Staining primary bovine satellite cells for Pax7, a common marker of satellite cells and myogenic potential. Protocol developed for https://www.thermofisher.com/antibody/product/PAX7-Antibody-Polyclonal/PA5-68506 (Thermo Fisher Pax7 antibody PA5-68506; rabbit IgG anti-Pax7)

Guidelines

For reference general volumes for given well formats are:

- 96-well = 100 uL
- 48-well = 150 uL
- 24-well = 300 uL
- 12-well = 500 uL
- 6-well = 750 uL

** recommended to use a PAP-pen to select a smaller region of 6-well plates after initial fixing / washing, as this will save antibody

Materials

MATERIALS

- 4% paraformaldehyde/1XPBS solution
- Goat-anti-rabbit-Alexafluor 488 **Thermo Fisher Scientific Catalog #**A11008
- PBS
- VECTASHIELD® Hardset™ Antifade Mounting Medium **Catalog #**H-1400
- PAX7 Polyclonal Antibody **Thermo Fisher Scientific Catalog #**PA5-68506
- Wash buffer (PBS / 5% goat serum / 0.05% NaAzide)
- Permeabilization solution (PBS / 0.5% Triton X-100)
- PBST (PBS 1:1000 Tween-20)
- Phalloidin 594 **Thermo Fisher Scientific Catalog #**A12381

Fixation and Permeabilization (1 hour)

1 Aspirate media from cells

2 add cold 4% PFA to cells (enough to cover cells or scaffolds)

3 Incubate at room temperature for 30 minutes 00:30:00

4 Wash 3x with room temperature PBS

- NOTE: at this point, can parafilm and leave in the fridge overnight (or up to 1 week) before staining

5 Aspirate PBS and add cold Permeabilization solution for 15 minutes 00:15:00

6 Wash 3x with cold PBST

Primary Stain (1 hour, overnight incubation)

7 Aspirate PBST and add cold Wash buffer for 45 minutes 00:45:00

> Note
>
> During soak, can move to step 8

8 Dilute primary antibodies in wash buffer and keep on ice (protected from light). For given antibody, use the following dilutions:

- anti-Pax7 (1:500)
- Phalloidin-594 (1:100)

note* prepare enough antibody solution for all conditions (a little extra is usually good to make sure there is enough)

9 After step 7 incubation, wash 3x with cold PBST

10 Add primary antibody solutions and incubate overnight at 4C (parafilm to avoid evaporation)

Secondary Stain (1.5 hours)

11 Wash 3x with cold PBST

12 Aspirate PBST and add cold Wash buffer for 15 minutes 00:15:00

> Note
>
> during soak, can move to step 13

13 Dilute secondary antibodies in wash buffer and keep on ice (protected from light). For given antibody, use the following dilutions:

- 488 goat-anti-rabbit (1:500)

note* prepare enough antibody solution for all conditions (a little extra is usually good to make sure there is enough)

STEP CASE

for 3D, when not using DAPI mounting media 3 steps

In the case where you're not planning to use a dapi mounting media (ie 3D constructs), prepare a DAPI solution in a suitable blocking buffer (ie Wash Buffer or a BSA-containing buffer), and use that to prepare antibody solutions, instead of plain wash buffer

14 After step 12 incubation, aspirate Wash buffer from cells, and add secondary antibody solutions. Incubate in the dark at room temperate for 60 minutes 01:00:00

15 Wash cells 3X with cold PBST, leaving the last wash to soak for 5 minutes 00:05:00

16 Aspirate PBST, and add DAPI mounting media. Cover with cover-slip, and image after 10 minutes 00:10:00

- Pax7 = green
- Actin cytoskeleton = red
- nuclei = blue