VERSION 1

FEB 11, 2019

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.xx3fpqn

Protocol Citation: Andrew Stout, Natalie Rubio 2019. Bovine satellite cell Pax7 ICC. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.xx3fpqn>

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

Created: Feb 09, 2019

Last Modified: Feb 11, 2019

PROTOCOL integer ID:
 20187

Keywords: Staining, Immunocytochemistry

Bovine satellite cell Pax7 ICC V.1

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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

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⊗ 4% paraformaldehyde/1XPBS solution **Contributed by users**

⊗ Goat-anti-rabbit-Alexafluor 488 **Thermo Fisher Scientific Catalog #A11008**

⊗ PBS **Contributed by users**

⊗ VECTASHIELD® Hardset™ Antifade Mounting Medium **Contributed by users Catalog #H-1400**

⊗ PAX7 Polyclonal Antibody **Thermo Fisher Scientific Catalog #PA5-68506**

⊗ Wash buffer (PBS / 5% goat serum / 0.05% NaAzide) **Contributed by users**

⊗ Permeabilization solution (PBS / 0.5% Triton X-100) **Contributed by users**

⊗ PBST (PBS 1:1000 Tween-20) **Contributed by users**

⊗ Phalloidin 594 **Thermo Fisher Scientific Catalog #A12381**

STEP MATERIALS

⊗ 4% paraformaldehyde/1XPBS solution **Contributed by users**

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 13 includes a Step case.

for 3D, when not using DAPI mounting media

step case

for 3D, when not using DAPI mounting media

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

Step 16 includes a Step case.

for 3D, when not using DAPI mounting media