

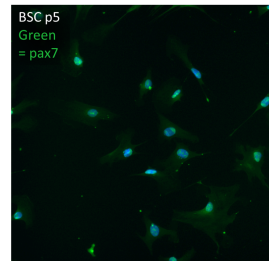
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Version 1

Bovine satellite cell Pax7 ICC V.1

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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**

STEP MATERIALS

- ⊗ 4% paraformaldehyde/1XPBS solution



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