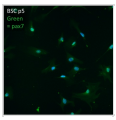

Bovine satellite cell Pax7 ICC

Version 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)

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

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

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

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

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

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

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 case

for 3D, when not using DAPI mounting media

If not using DAPI mounting media, and already included it in the secondary stains, leave constructs in final PBST wash and take directly for imaging