ABIN957814 – COL1 antibody, IF

Validation report #103250

Validation lab name:

Musculoskeletal Gene Therapy Research Laboratory, Mayo Clinic

Validating lab URL:

https://www.mayo.edu/research/labs/musculoskeletal-gene-therapy-research

Lot number:

113240

Positive control:

Rabbit tendon: COL1 is the most abundant collagen in tendons

Negative control:

Autofluorescence/endogenous tissue background staining control. Before applying the primary antibody, the rabbit tissues were inspected under the microscope using fluorescence illumination to ensure there was no signal inherent to the tissue itself. Rabbit tissues showed weak endogenous autofluorescence.
Rabbit cartilage: in normal cartilage, type II collagen represents 90% to 95% of the collagen, whereas COL1 expression is almost negligible.
Secondary antibody only control
Mouse IgG1 kappa isotype control

Protocol:

- Cut formalin-fixed paraffin-embedded (FFPE) rabbit tissues into 5µM sections using a microtome.
- Deparaffinize and rehydrate by immersing the slides through the following solutions
  - Xylene, wash sections 3x 5min each
  - 100% Ethanol, wash sections 2x 3min each
  - 95% Ethanol, wash sections 2x 1min each

    - 70% Ethanol, wash sections 2x 1min each
    - Deionized Water, wash sections 2x for 5 min each
- Protease-induced epitope retrieval protocol (0.1%hyaluronidase with 0.1% pronase in phosphate-buffered saline, PBS) using a microwave tissue processor (Milestone) set to 37°C for 30min.
- Wash the sections by immersing them in distilled water for 5min.
- Incubate tissue sections with 5% normal goat serum (Abcam, ab7481) in PBS containing 1% Tween 20 (PBST) for 30min at RT.
- Blot excess serum from the section but do not rinse**.**
- Incubate sections with Streptavidin-Biotin blocking solution (SP-2002, Vector Laboratories, Inc) for 15min following manufacturer's recommendations.
- Incubate sections with
    - 100µL primary mouse anti-Collagen type I (COL1) antibody (antibodies-online, ABIN957814, lot 113240) diluted 1:50 in PBST ON at 4°C.
    - Mouse IgG1 kappa isotype control antibody (Abcam, Ab18443) diluted 1:50 in PBST ON at 4°C.
- Wash sections 3x with PBS-T for 5min each.
- Add the secondary biotinylated goat anti-mouse IgG1 antibody (NBP1-69914B, Novus Biologicals) incubated in a humidified chamber at RT for 1h.
- Wash sections 3x with PBS-T for 5min each.
- Tap off excess wash buffer.
- Incubate sections in fluorescein-streptavidin conjugate (Vector Laboratories Inc., SA-5001) for 5min at RT in a dark slide moat.
- Wash sections 3x with PBS-T for 5min each.
- Incubated sections with biotinylated anti-streptavidin antibody (Vector Laboratories Inc., BA-0500) for 30min at RT in a dark slide moat.
- Incubate the sections in fluorescein-streptavidin conjugate (Vector Laboratories Inc., BA-0500, SA-5001) for 5min at RT in a dark slide moat.
- Wash sections 3x with PBS-T for 5min each.
- Apply one drop of Vectashield anti-fade mounting medium with DAPI (Vector Laboratories, Inc, H-1200) and coverslip.
- Slides can be stored at 4°C in a dark slide box until microscope analysis to prevent loss of fluorescence.
- Examine slides under a fluorescence automated inverted microscope (Olympus, IX83 microscope).

Experimental Notes

The aim was to demonstrate the performance characteristics of collagen type I (COL1) protein expression using ABIN957814 on FFPE rabbit tissues by using

immunofluorescence and that the performance characteristics of the antibody is suitable for the intended analytical use.

We decided to use a biotinylated secondary antibody in conjunction with strept-/avidin and a biotinylated reporter in order to enhance the fluorescence signal usingthe biotinylated goat anti-mouse IgG1 secondary antibody (NBP1-69914B, Novus Biologicals, CO). This technique provided an increased fluorescence intensity (see attached image files).

ABIN957814 specifically labeled the target antigen using IF on FFPE rabbit tendon. No signal was detected in negative control tissue (normal rabbit cartilage), isotype control and the secondary antibody only control.

We also compared ABIN957814 against another collagen type I antibody from a different company and under similar conditions. ABIN957814 antibody seemed to provide superior immunofluorescence signal.

In addition , FFPE sections of normal rabbit condyle were stained using ABIN957814. Normal rabbit cartilage showed no immunofluorescence signal for collagen type 1. The expected COL 1 staining at the subchondral bone was observed.

Notes

The COL1 antibody ABIN957814 specifically labels the target antigen in IF on FFPE rabbit tendon.

Figure legend

1 Collagen type I protein expression in Rabbit tendon. Formalin-fixed paraffin embedded sections of normal rabbit tendon was stained with ABIN957814 or an isotype control antibody (A, B; green) and mounted in mounting medium with DAPI (B, E; blue). Collagen fibers arranged in tightly cohesive parallel and well-dermacated bundles that contain flatted nuclei of tenocytes (C, F; merged). Magnification: 10X. 2 FFPE sections of normal rabbit condyle stained with ABIN957814. Normal rabbit cartilage (red lines) show no immunofluorescence signal for collagen type 1 while the expected COL11 staining (green) at the subchondral bone (white arrow) was observed.

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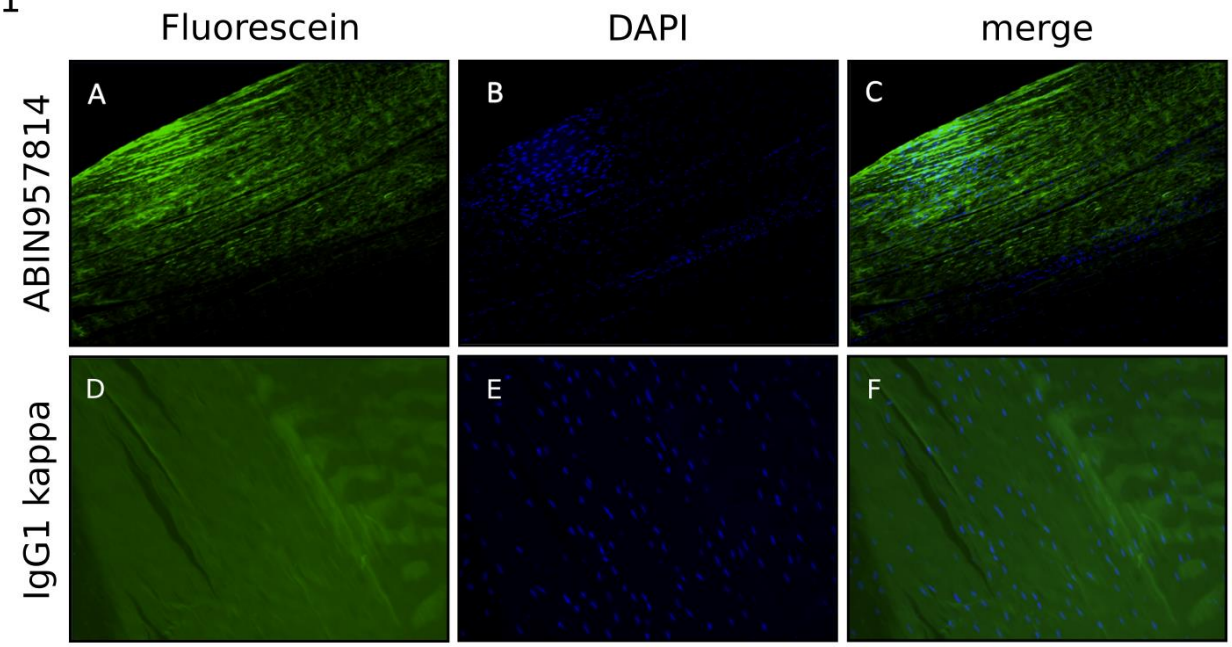
Fluorescein
DAPI
merge
ABIN957814
IgG1 kappa
A
B
C
D
E
F

2


Cartilage
Subchondral
Condyle