

Materials and Methods

Experimental Strategy

The histology platform APEX evaluated two recombinant Fc Rabbit anti-Dystrophin antibodies on formalin-fixed paraffin-embedded (FFPE) biceps femoris sections from mouse (APEX ID 2400831), rat (APEX ID 2000500) and dog (APEX ID 2500210).

Initially, various conditions of pretreatment and antibody concentrations were tested on each species and for each antibody to determine optimal labelling parameters.

Antibody performance was assessed based on biological relevance (sub sarcolemic localization), staining intensity, specificity, and background signal. No reference antibody was available.

Biological Material

- All formalin-fixed paraffin-embedded (FFPE) samples were already available on the platform. No animal has been sacrificed for these tests.
- Routinely processed for paraffin embedding (*VWR, 10048502*)
- Serial 4 µm-thick fresh sections were cut out of each block and immunolabelled with the anti-Dystrophin primary antibodies.

Antibody Testing

The tested recombinant antibodies were:

- Abcam; ref ab315102 = anti-Dystrophin [RM1069] Rabbit Multiclonal Ab (170 €/20 µL)
- Cell Signaling Technology, ref #47759 = anti-Dystrophin (E4F3S) Rabbit Monoclonal Ab (245 €/20 µL)

The recombinant anti-Dystrophin antibodies were tested under these conditions:

Pretreatment :

1. CC1 Tris-EDTA pH 8 (*Roche diagnostics, 950-500*) at 98°C for 40 min
2. CC2 Citrate pH 6 (*Roche diagnostics, 950-223*) at 91°C for 40 min or with protease

Tested dilutions :

- Abcam Ab16669 : 1:100 1:200 and 1:400
- Ozyme #85061 : 1:50, 1:100 and 1:200

The antibodies were diluted in Discovery Ab Diluent (*Roche Diagnostics, 760-108*)
Saturation with goat serum 5% incubated for 32 min

Primary antibody incubated for 1 hour at 37°C

Peroxydase inhibition solution (*Roche diagnostics*, 760-159) incubated for 8 min

Secondary antibody: Omap rabbit HRP incubated for 16 min (*Roche Diagnostics* 760-4311)

Revelation DAB (*Fisher TA-125-QHDX*) for 20 min

Counter staining Hematoxilin and bluing agent from Roche diagnostics incubated for 20 and 4 min (790-2208 /760-2037)

Image Acquisition

Images were acquired using a scanner Zeiss Axioscan Z1.

Results Summary

The Abcam antibody was validated on mouse and rat tissues.

The CST/Ozyme antibody was validated for all three species : mouse, rat and dog.

Validated conditions :

	Dilution	Pretreatment
Abcam ab315102	1:100	CC1 or CC2
CST/Ozyme #47759	1:30	CC1 or CC2

Biceps femoris sections of mouse

Anti-Dystrophin ab315102 (Abcam)
Recombinant multiclonal
(1:100)



=> Staining +, subsarcolemmic, no background signal

Biceps femoris sections of rat

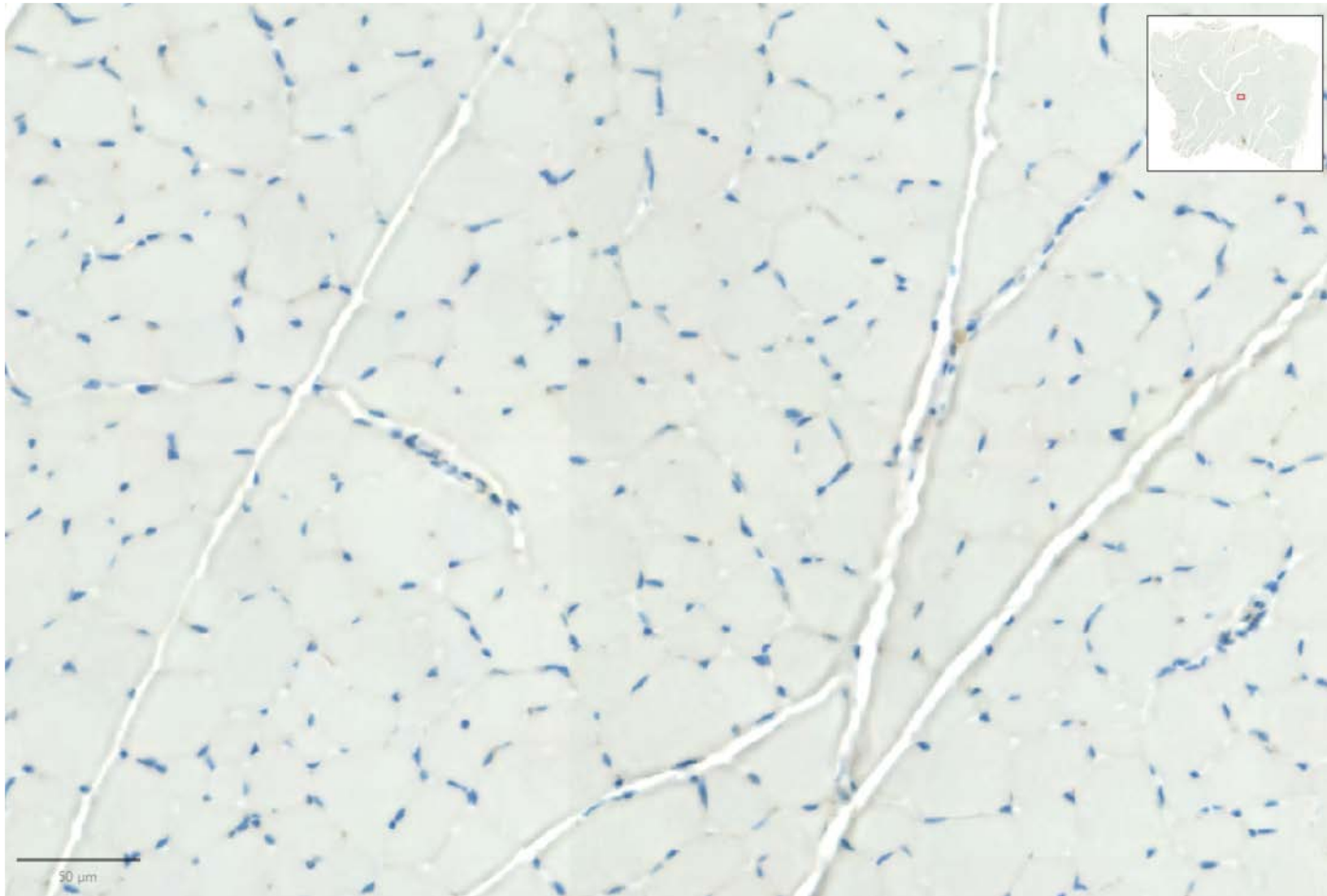
Anti-Dystrophin ab315102 (Abcam)
Recombinant multiclonal
(1:100)



=> Staining +, subsarcolemmic, no background signal

Biceps femoris sections of dog

Anti-Dystrophin ab315102 (Abcam)
Recombinant multiclonal
(1:100)



=> No staining

Biceps femoris sections of mouse

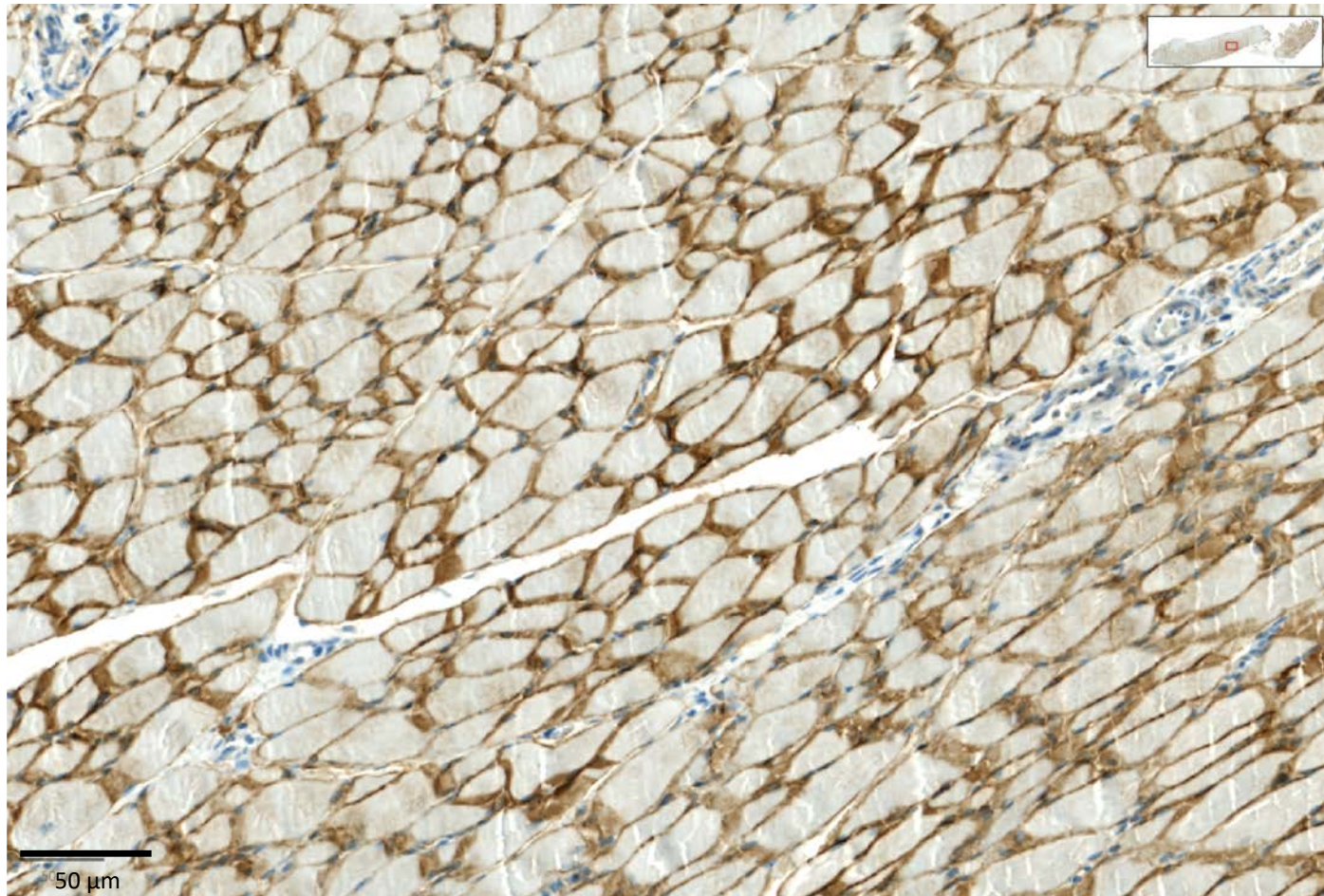
Anti-Dystrophin 47759S (CST)
Recombinant monoclonal
(1:30)



=> Staining +, subsarcolemmic, no background signal

Biceps femoris sections of rat

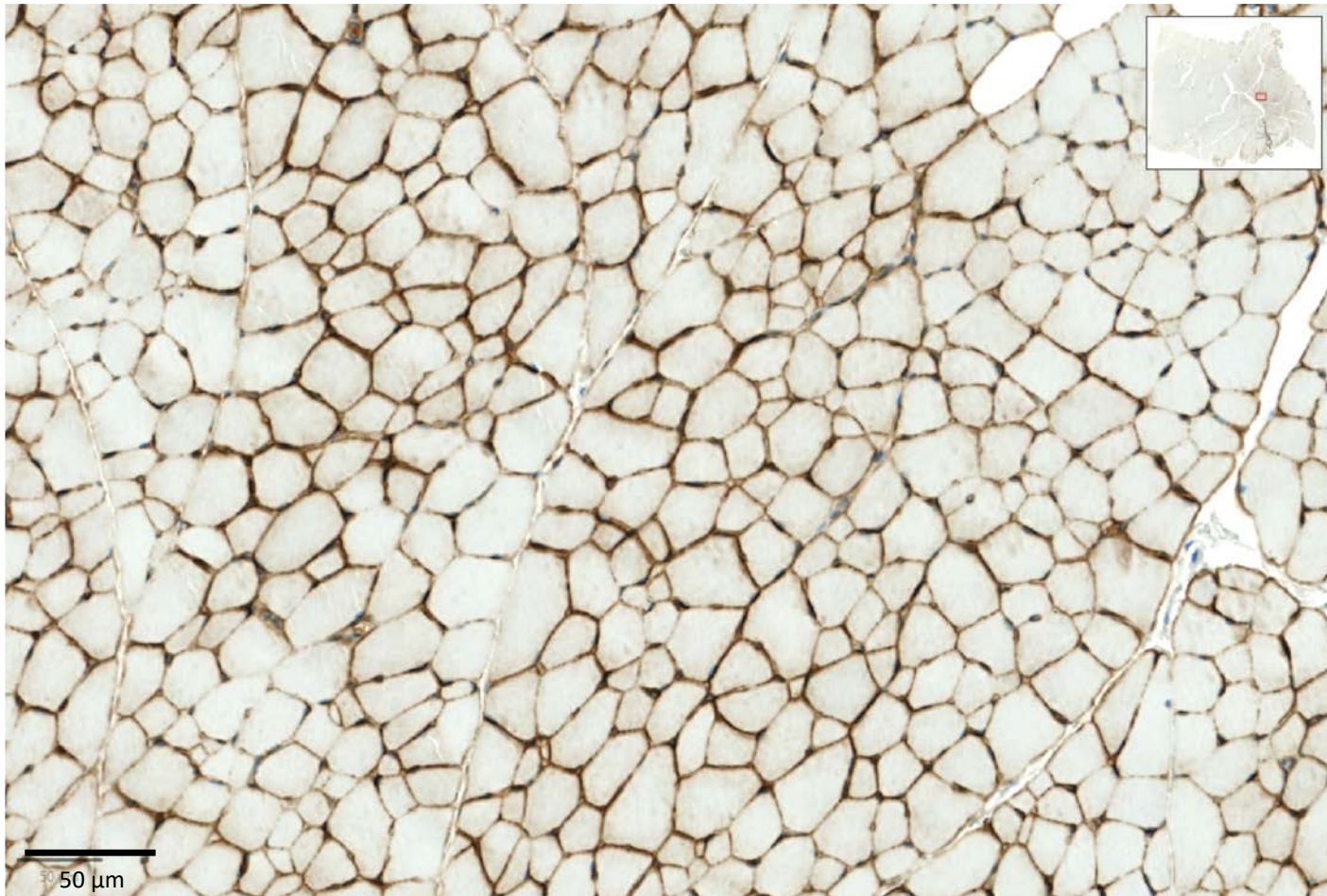
Anti-Dystrophin 47759S (CST)
Recombinant monoclonal
(1:30)



=> Staining +, subsarcolemmic, no background signal

Biceps femoris sections of dog

Anti-Dystrophin 47759S (CST)
Recombinant monoclonal
(1:30)



=> Staining +, subsarcolemmic, no background signal