

## Materials and Methods

### Experimental Strategy

The histology platform APEX evaluated three recombinant Fc Rabbit anti-Laminin 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 localization, staining intensity, specificity, and background signal. A reference antibody served for comparative validation.

### 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-Laminin primary antibodies.

### Antibody Testing

The tested recombinant antibodies were:

- Abcam; ref ab315275 = anti-Laminin  $\alpha$ 1 [EPR27259-172] Rabbit mAb (222 €/20 µL)
- ABCD antibodies, ref ABCD\_AB628 = anti-Laminin  $\alpha$ 1 Rabbit mAb (220 €/100 µg)
- Absolute antibody, ref Ab02770 = anti-Laminin  $\gamma$  Rabbit mAb (460 €/200 µg)

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

Pretreatment :

1. **CC1** Tris-EDTA pH 8 (*Roche diagnostics, 950-500*) at 95°C for 40 min
2. **CC2** Citrate pH 6 (*Roche diagnostics, 950-223*) at 91°C for 40 min

Tested dilutions :

- Abcam Ab315275 : 1:50
- ABCD AB628 : 1:50
- Absolute Antibody : 1 :50

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*, 760-4307) 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)

A reference antibody was used as a control: Sigma L9393 anti-Laminin  $\alpha$ 1 Rabbit polyclonal (1:30)

## **Image Acquisition**

Images were acquired using a scanner Zeiss Axioscan Z1.

## **Results Summary**

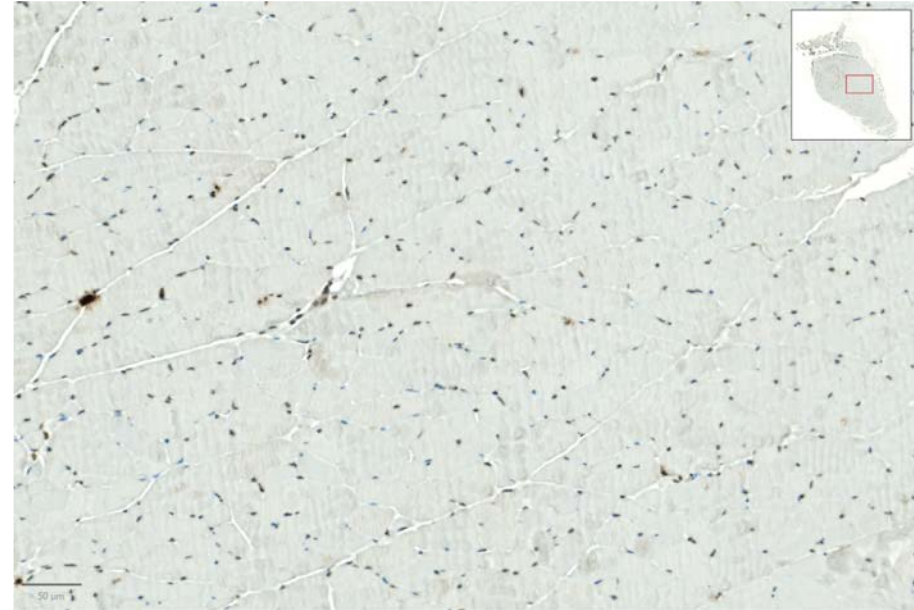
None of the tested recombinant antibodies gave signal under the tested conditions.

# Coupes de biceps fémoral de souris

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



**Anti-Laminine ABCD\_AB628 (ABCD Antibodies)**  
**Recombinant monoclonal**  
(1:50)



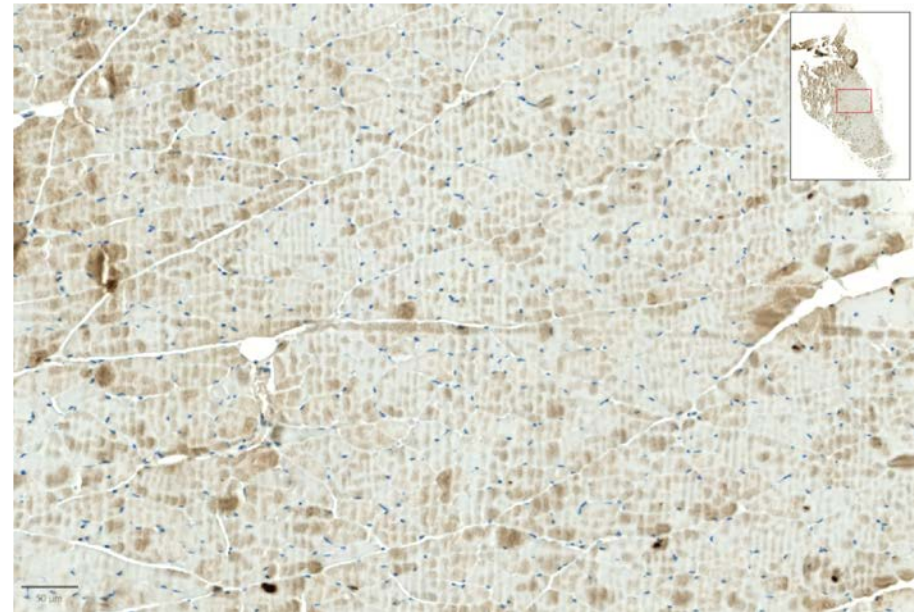
⇒ Pas de marquage

# Coupes de biceps fémoral de souris

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



**Anti-Laminine Ab315275 (Abcam)**  
**Recombinant monoclonal**  
(1:50)



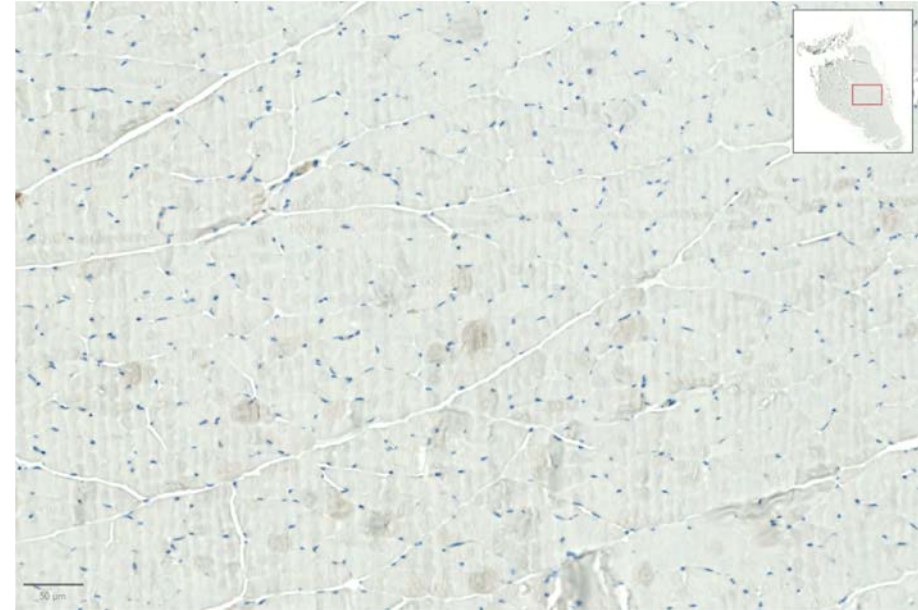
⇒ Pas de marquage,  
présence de bruit de fond

# Coupes de biceps fémoral de souris

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



**Anti-Laminine Ab02770-23.0 (Absolute Antibody)**  
**Recombinant monoclonal**  
(1:50)



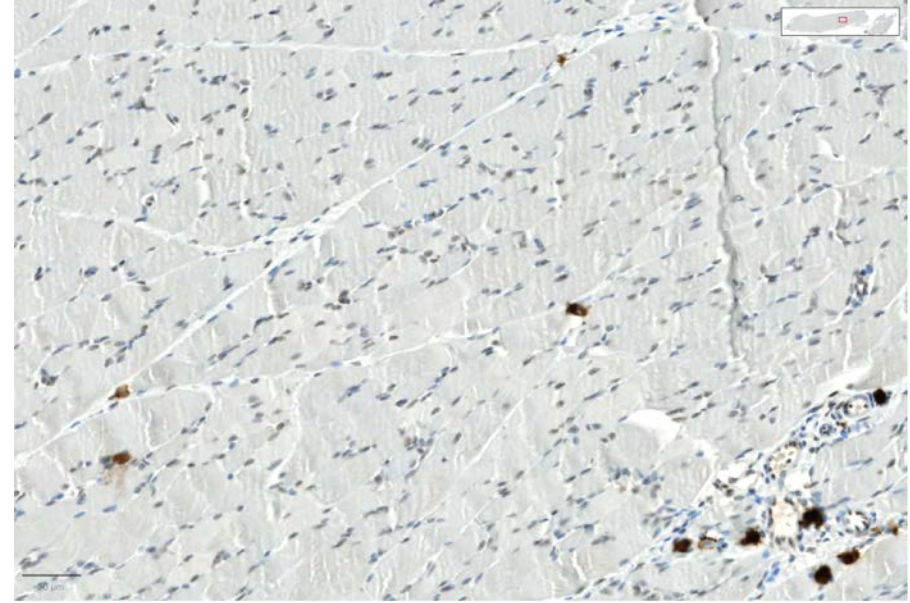
⇒ Pas de marquage

# Coupes de biceps fémoral de rat

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



**Anti-Laminine ABCD\_AB628 (ABCD Antibodies)**  
**Recombinant monoclonal**  
(1:50)



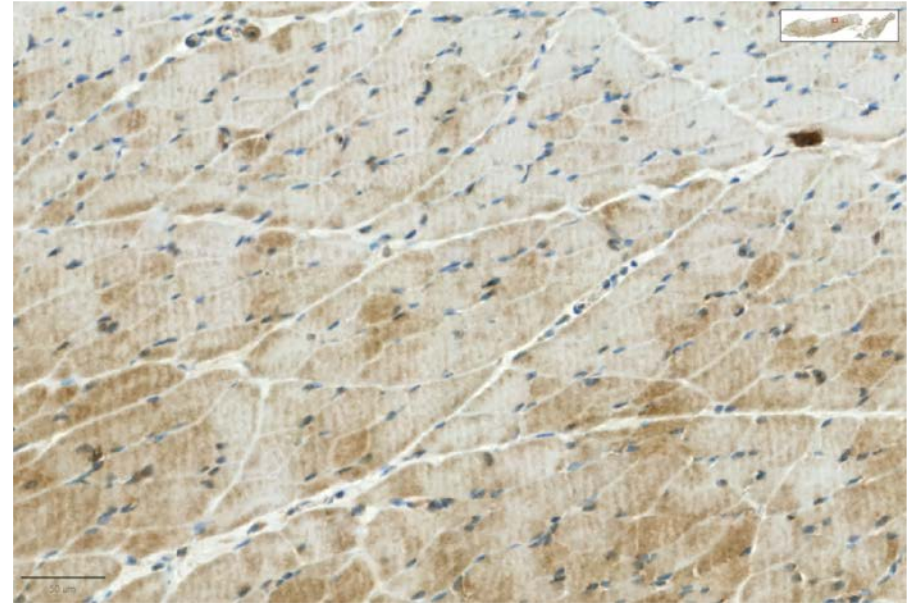
⇒ Pas de marquage

# Coupes de biceps fémoral de rat

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



**Anti-Laminine Ab315275 (Abcam)**  
**Recombinant monoclonal**  
(1:50)



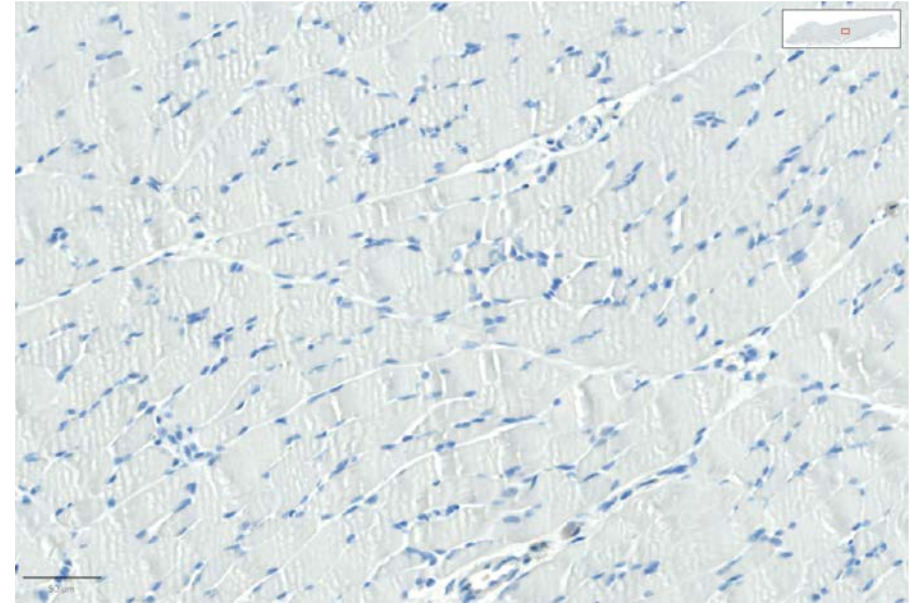
⇒ Pas de marquage,  
présence de bruit de fond

# Coupes de biceps fémoral de rat

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



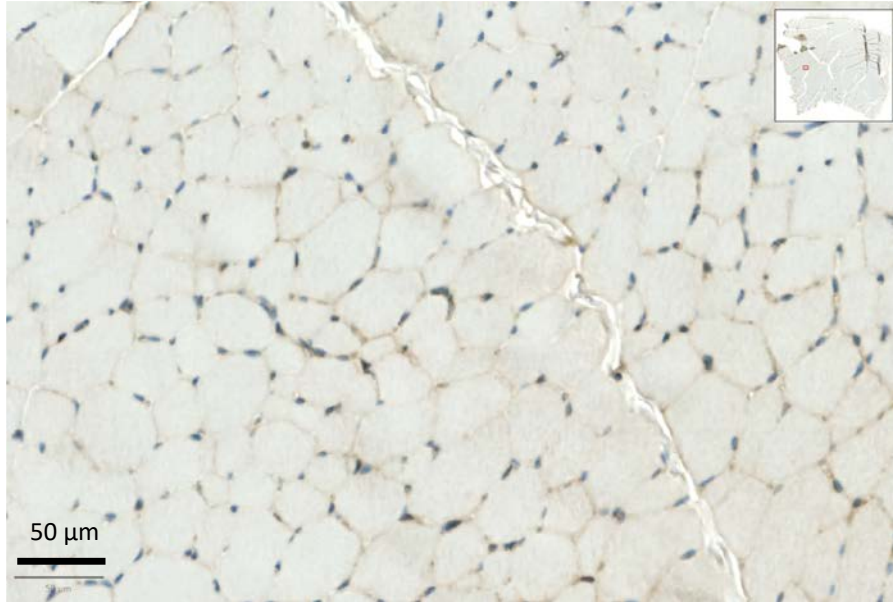
**Anti-Laminine Ab02770-23.0 (Absolute Antibody)**  
**Recombinant monoclonal**  
(1:50)



⇒ Pas de marquage

# Coupes de biceps fémoral de chien

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



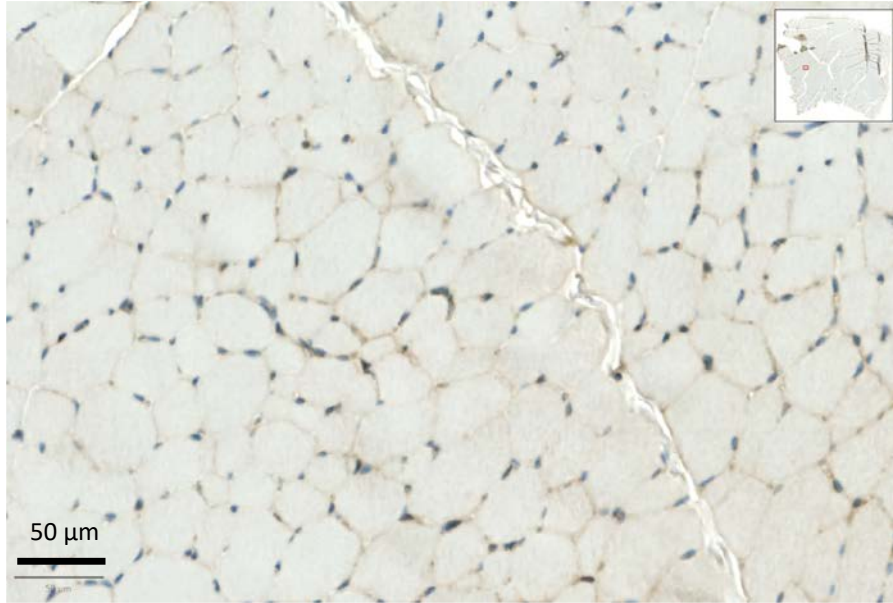
**Anti-Laminine ABCD\_AB628 (ABCD Antibodies)**  
**Recombinant monoclonal**  
(1:50)



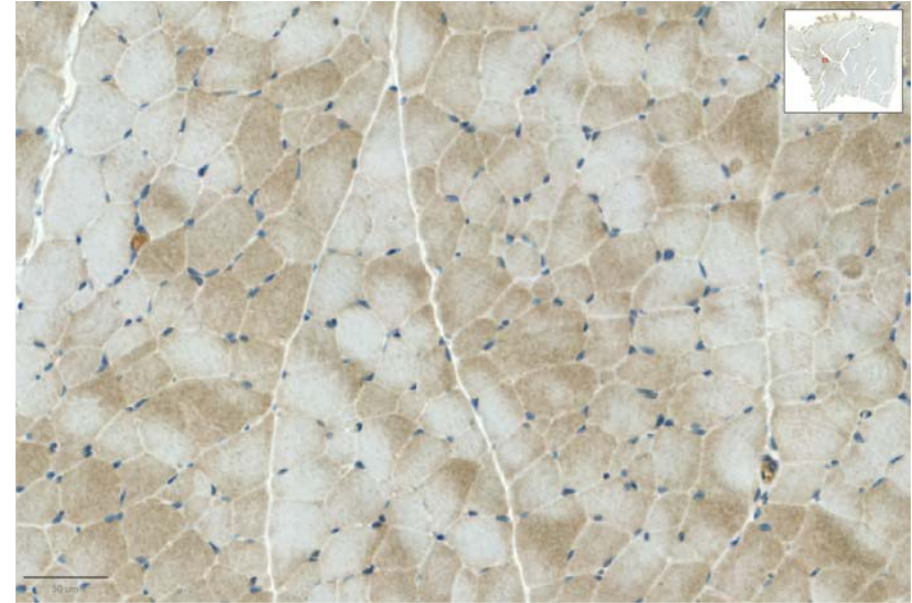
⇒ Pas de marquage

# Coupes de biceps fémoral de chien

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



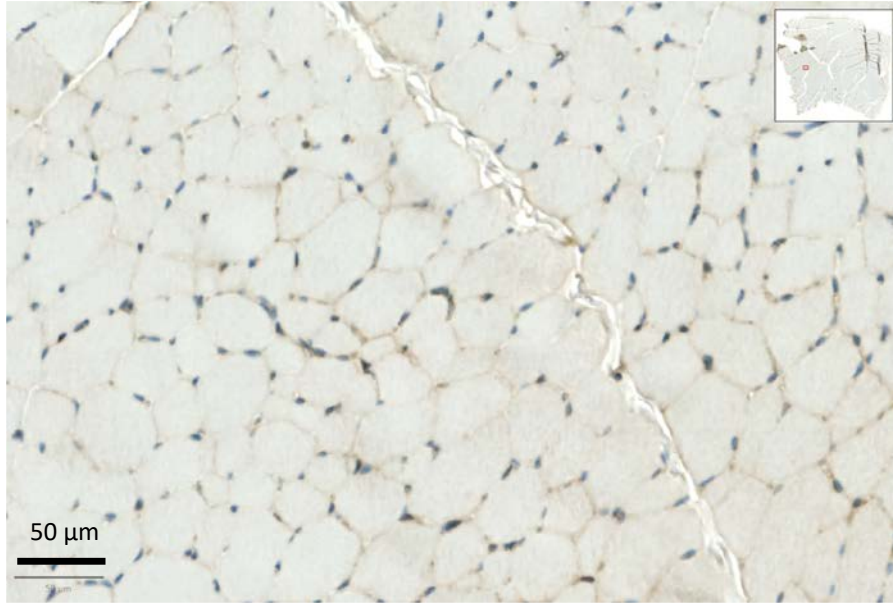
**Anti-Laminine Ab315275 (Abcam)**  
**Recombinant monoclonal**  
(1:50)



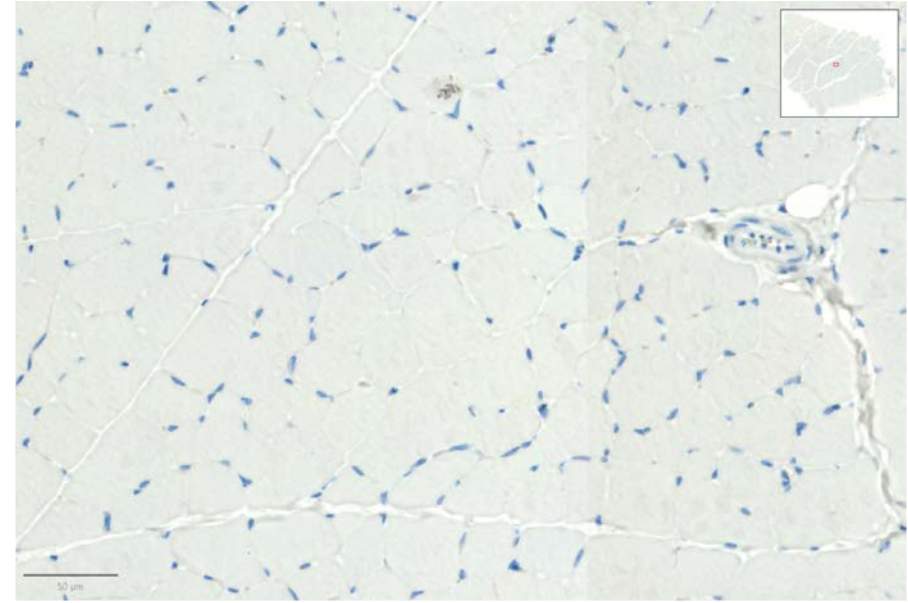
⇒ Pas de marquage,  
présence de bruit de fond

# Coupes de biceps fémoral de chien

**Anticorps Anti-Laminine**  
**Polyclonal de référence**  
(1:30)



**Anti-Laminine Ab02770-23.0 (Absolute Antibody)**  
**Recombinant monoclonal**  
(1:50)



⇒ Pas de marquage