Materials and Methods

Experimental Strategy

The histology platform APEX evaluated four recombinant Fc Rabbit anti-CD3 antibodies on formalin-fixed paraffin-embedded (FFPE) spleen sections from C57Bl6 mouse (APEX ID 2400674), Fischer rat (APEX ID 2400601 & 2400534) and *Macaca fascicularis* (APEX ID 2400829).

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 staining intensity, specificity, and background signal compared to a reference polyclonal anti-CD3 antibody used by the platform.

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 sections were cut
- For each paraffin blocs, 5 serial sections 4 µm-thick were immunolabelled with primary antibodies against CD3

Antibody Testing

The tested antibodies were:

- Abcam; ref ab16669 = anti-CD3ε (SP7) Rabbit mAb (130 €/20 µL)
- ABCD antibodies, ref ABCD_AD036 = anti-CD3ε Rabbit mAb (220 €/100 µg)
- Absolute antibody, ref YTH12.5 = anti-CD3ε Rabbit mAb (460 €/200 µg)
- Ozyme, ref #85061 = anti-CD3ε (D7A6E™) XP® Rabbit mAb (229 €/20 µL)

The recombinant anti-CD3 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 Ab16669 : 1:150 and 1:300
- ABCD AD036 : 1:100 and 1:200
- Absolute YTH 12.5 1:100 and 1:200
- Ozyme #85061 : 1:200 and 1:400

The antibodies were diluted in Discovery Ab Diluent (*Roche Diagnostics, 760-108)*

Blocking solution from Diagomics *(110050)* incubated for 32 min

Primary antibody incubated for 1 hour at 37°C

Peroxydase inhibition solution *(Roche diagnostics, kit 760-4307)* incubated for 8 min

Secundary antibody: Goat anti-rabbit IgG(H+L) biotin 1:200 32 min at 37°C *(E0432)*

Revelation with Strepta PER *(Invitrogen, 434323)* and *DAB (Roche diagnostics, kit 760-4304)* for 32 min

Counter staining Hematoxilin II *(790-2208)* and bluing agent *(760-2037)* from Roche diagnostics incubated for 8 and 4 min.

A reference antibody was used as a control: Dako ref A0452 = polyclonal Rabbit IgG (1:200) ; with CC1 pretreatment.

Image Acquisition

Images were acquired using a scanner Zeiss Axioscan Z1.

Results Summary

Two recombinant antibodies were validated. Two antibodies did not give signal under the tested conditions.

The Ozyme antibody displayed better immunolabelling properties compared to the previous reference antibody (Dako polyclonal rabbit Ab #A0452).

Validated conditions :

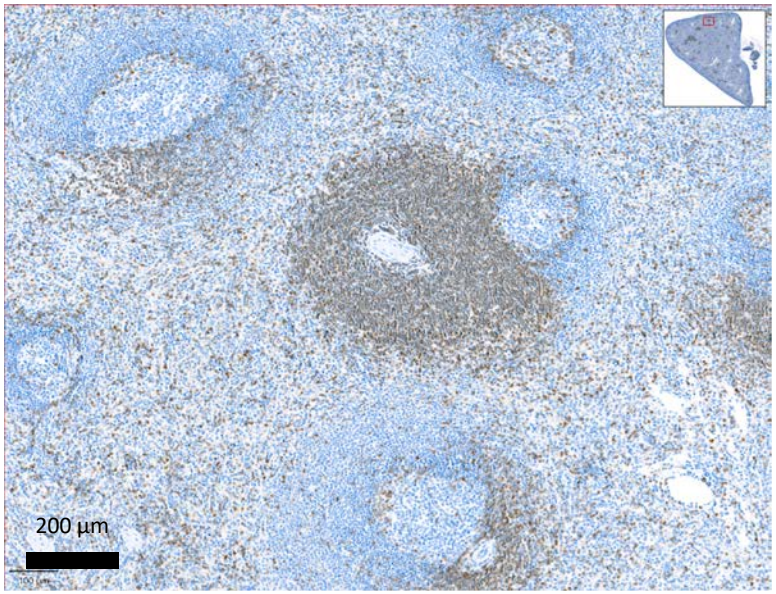
	Dilution	Pretreatment
Abcam Ab16669	1:150	CC1 or CC2
ABCD AD036	/	/
AbAb YTH12.5	/	/
Ozyme Y85061	1 :150 to 1:50	CC1 or CC2

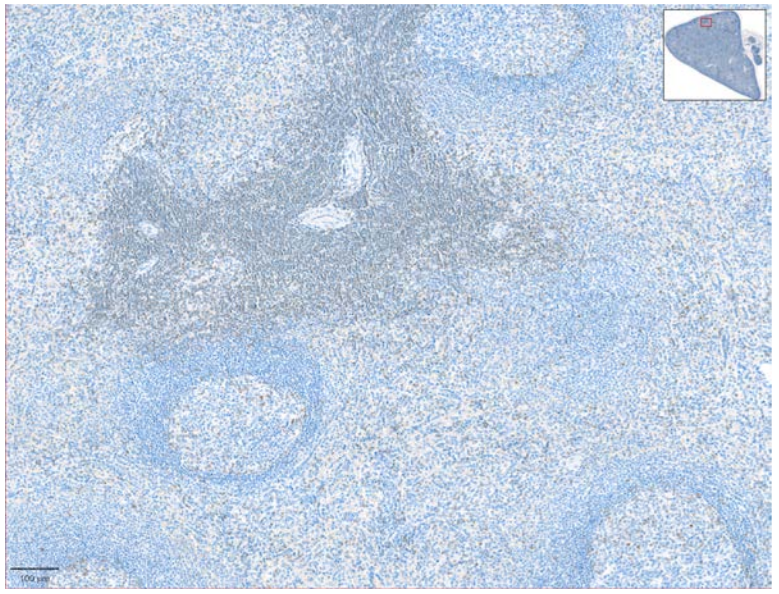
Coupes de rate de *Macaca fascicularis*

Anti-CD3 polyclonal de référence
(1:2000)

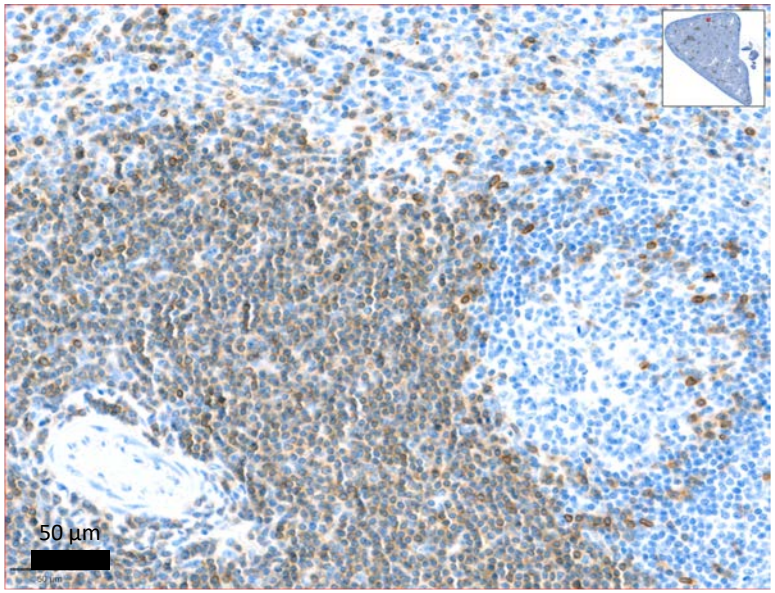
Anti-CD3 Ab16669** (Abcam)**
Recombinant monoclonal
(1:150; CC1)

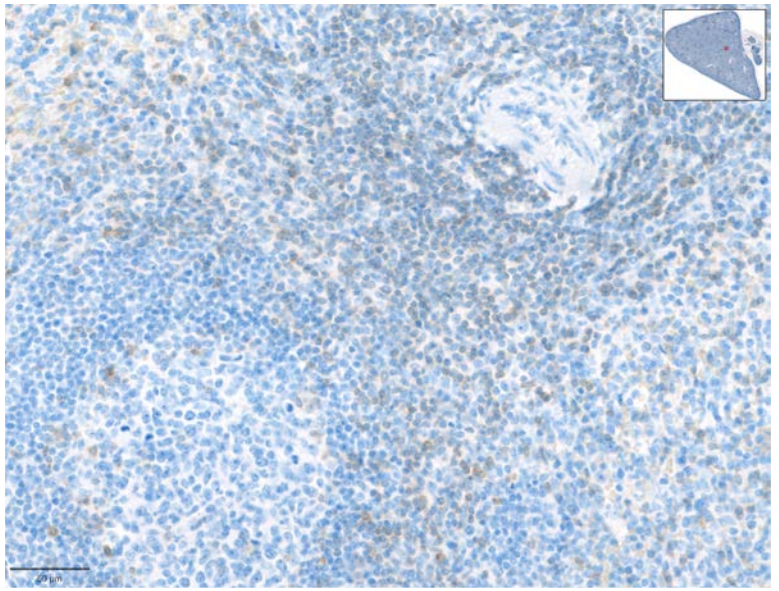
x 2.5





x 11.7



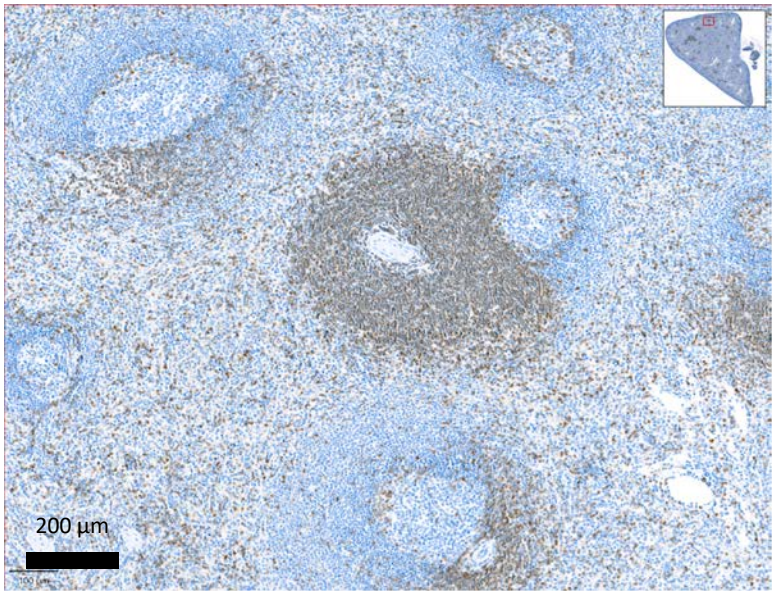


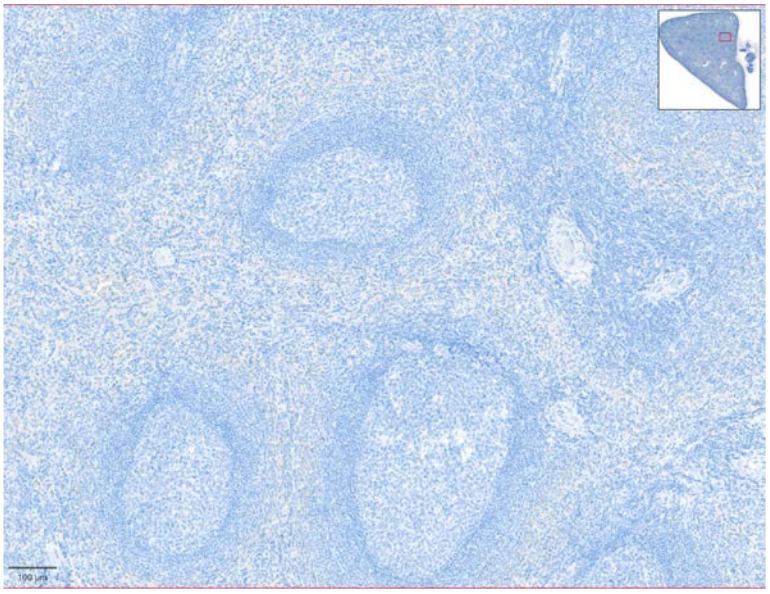
Coupes de rate de *Macaca fascicularis*

Anti-CD3 polyclonal de référence
(1:2000)

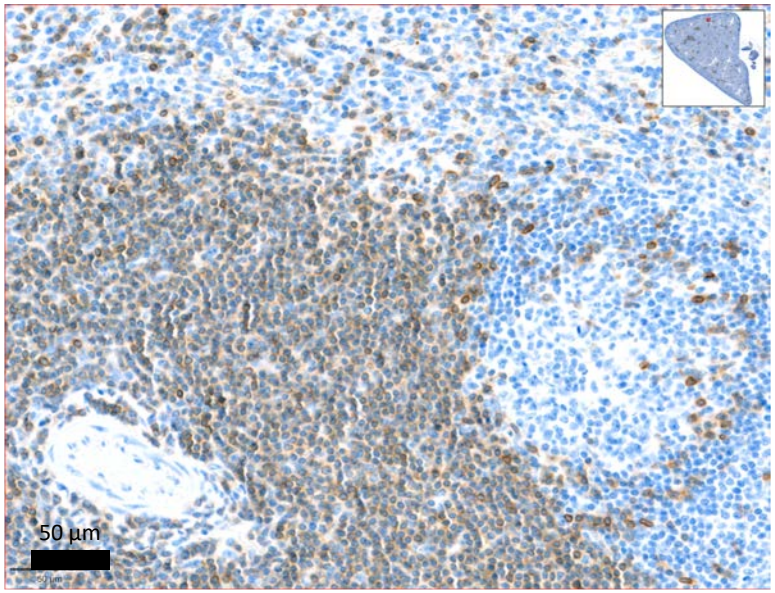
Anti-CD3 AD036 (ABCD)
Recombinant monoclonal
(1:100; CC2)

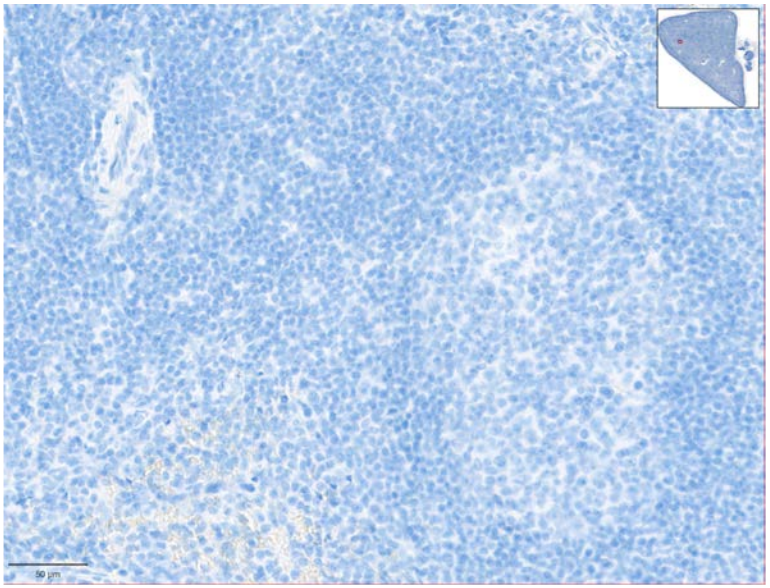
x 2.5





x 11.7



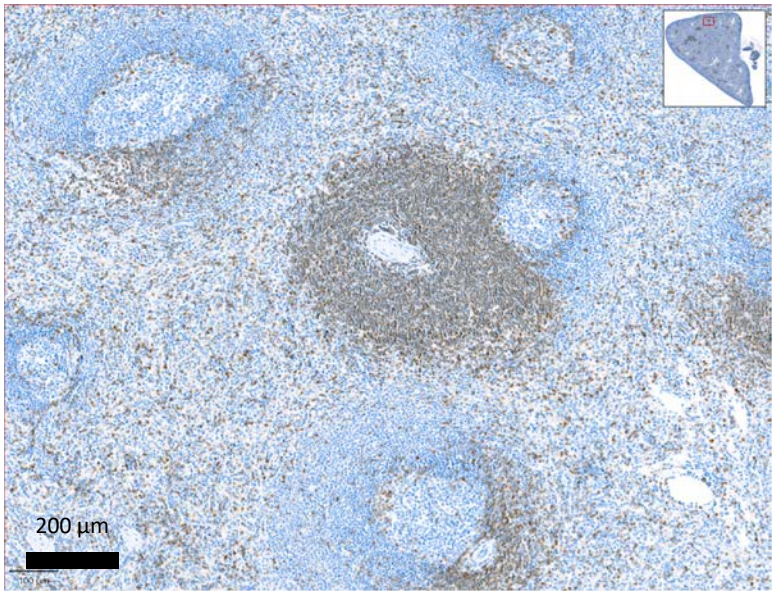


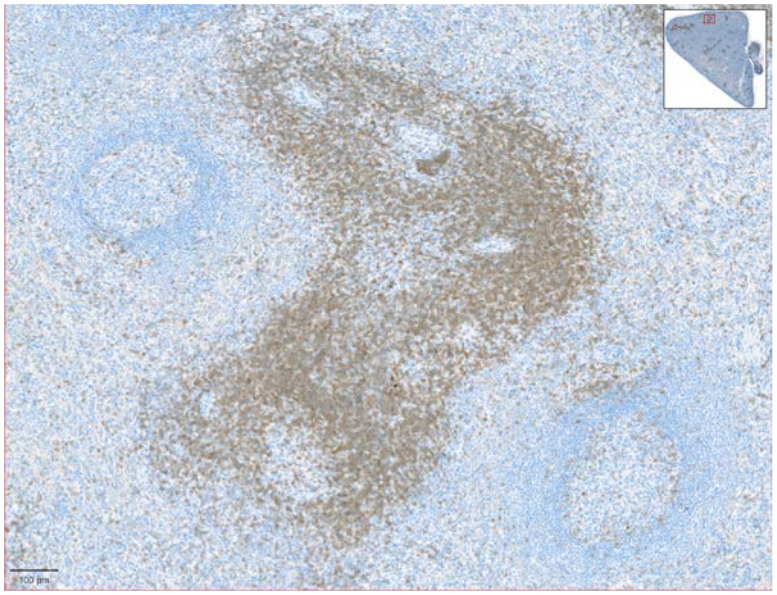
Coupes de rate de *Macaca fascicularis*

Anti-CD3 polyclonal de référence
(1:2000)

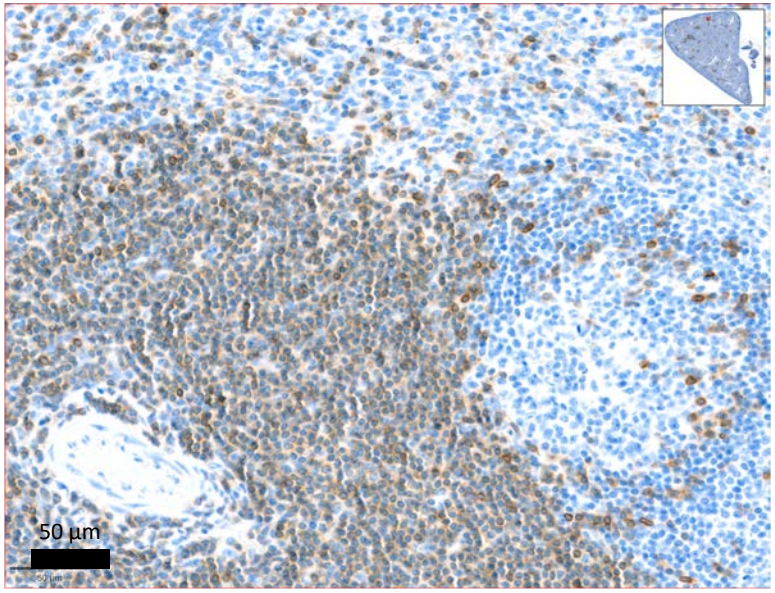
Anti-CD3 85061 (CST)
Recombinant monoclonal
(1:150; CC2)

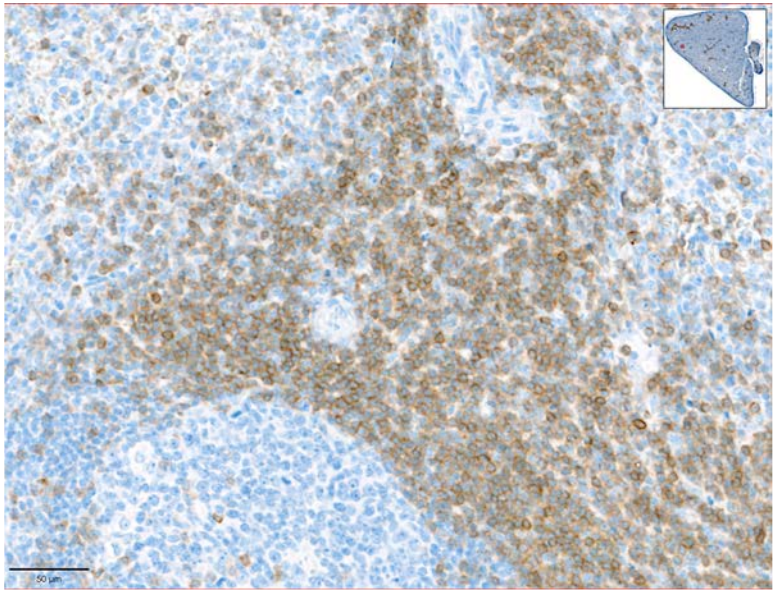
x 2.5





x 11.7



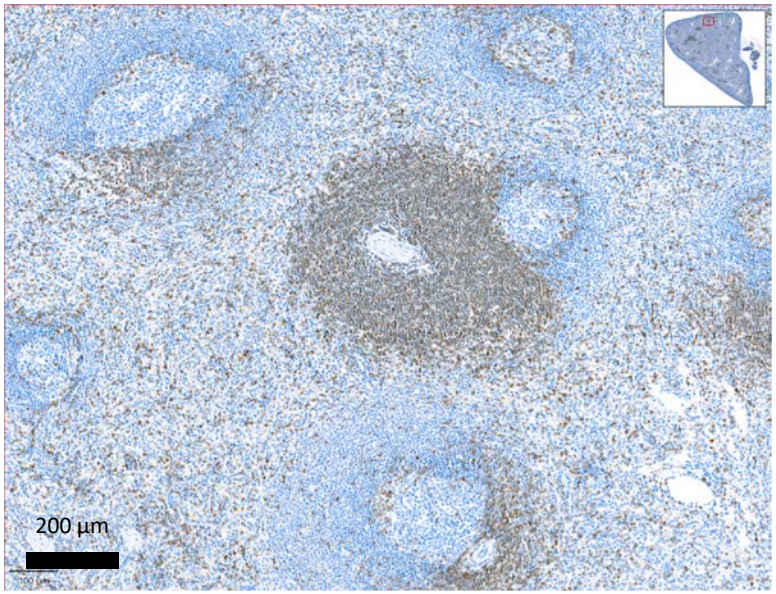


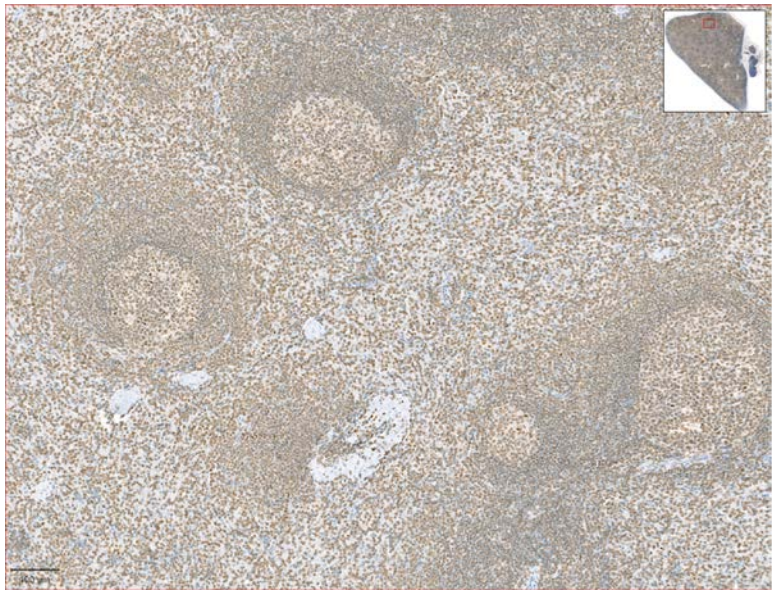
Coupes de rate de *Macaca fascicularis*

Anti-CD3 polyclonal de référence
(1:2000)

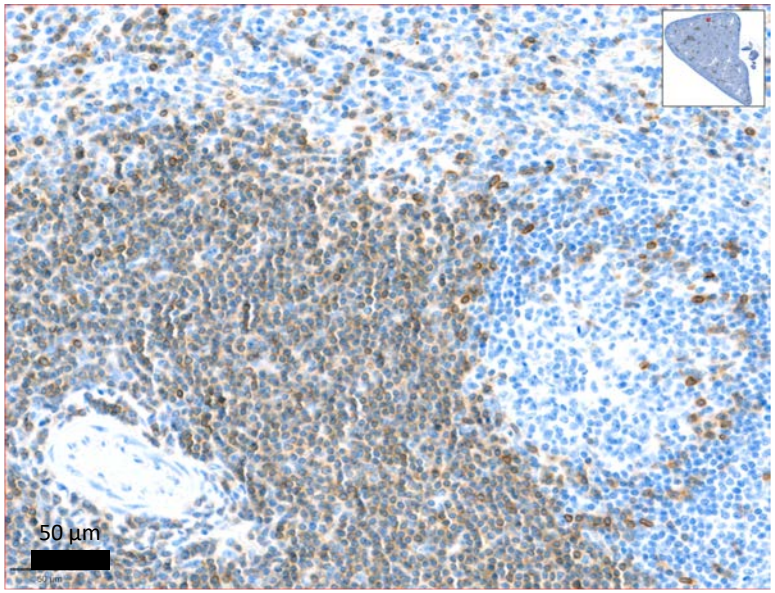
Anti-CD3 Yth12.5 (Absolute antibody)
Recombinant monoclonal
(1:100; CC2)

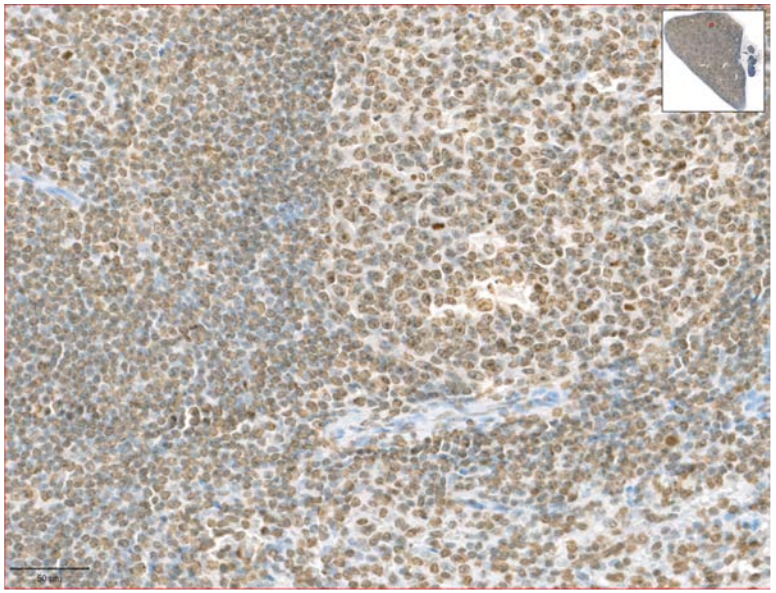
x 2.5





x 11.7



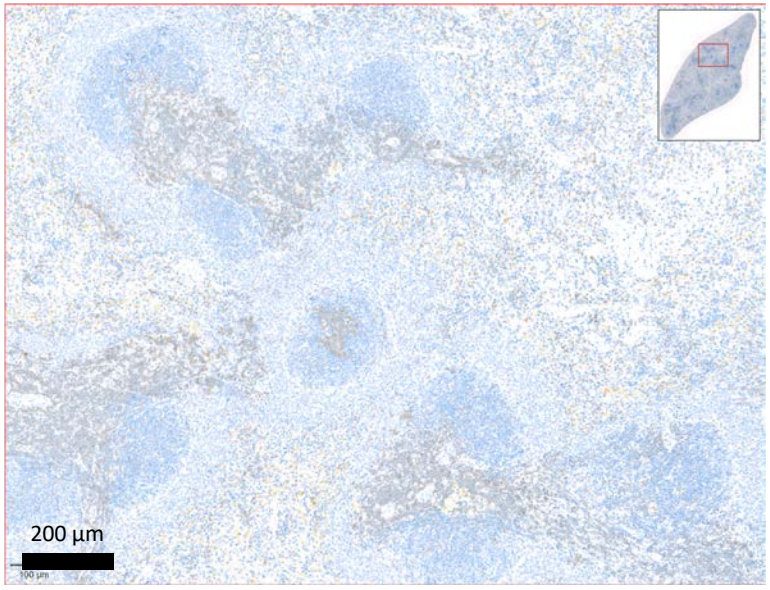


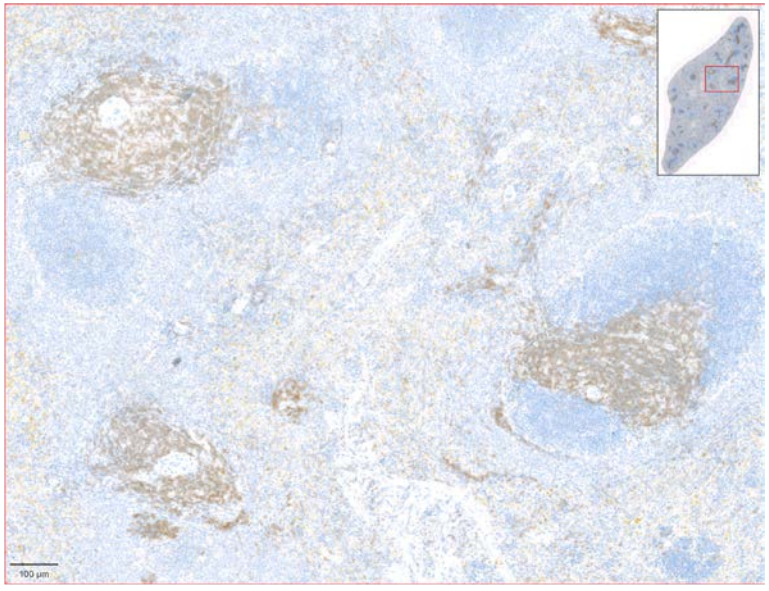
Coupes de rate de rat Fischer

Anti-CD3 polyclonal de référence
(1:200)

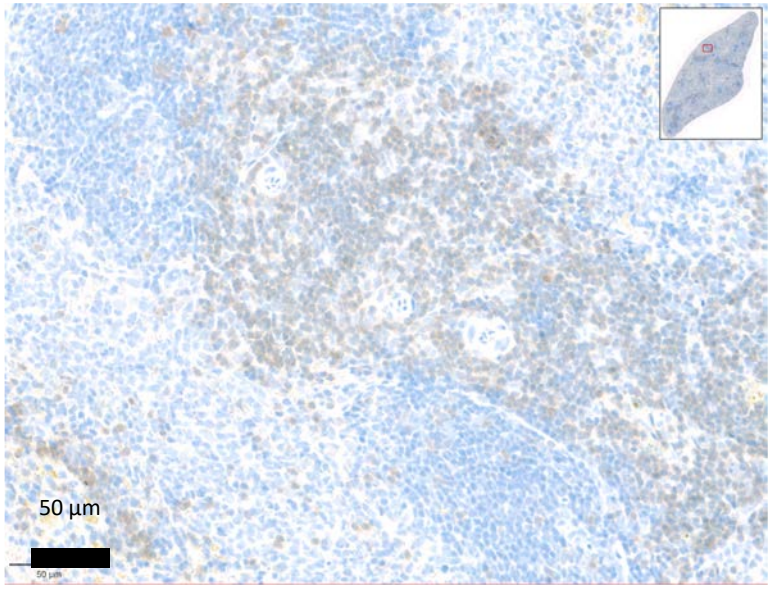
Anti-CD3 Ab16669** (Abcam)**
Recombinant monoclonal
(1:50; CC2 – *résultats similaires avec CC1*)

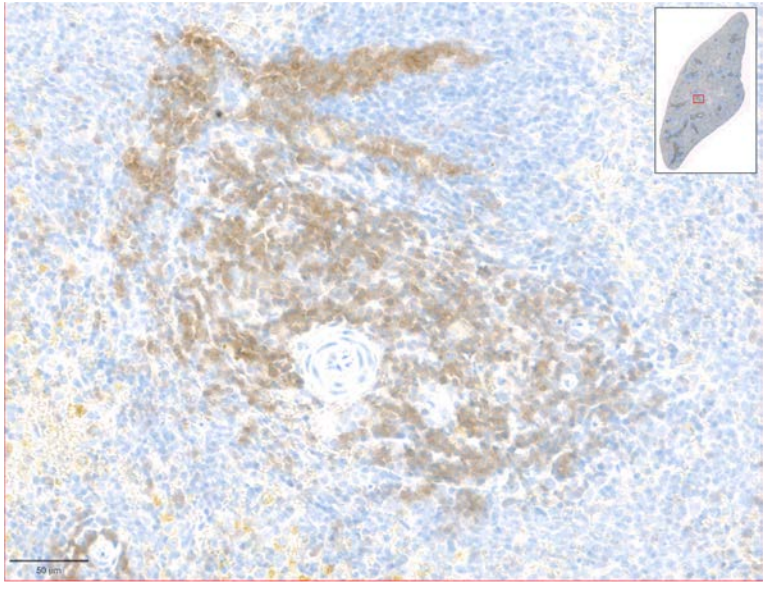
x 2.5





x 11.7



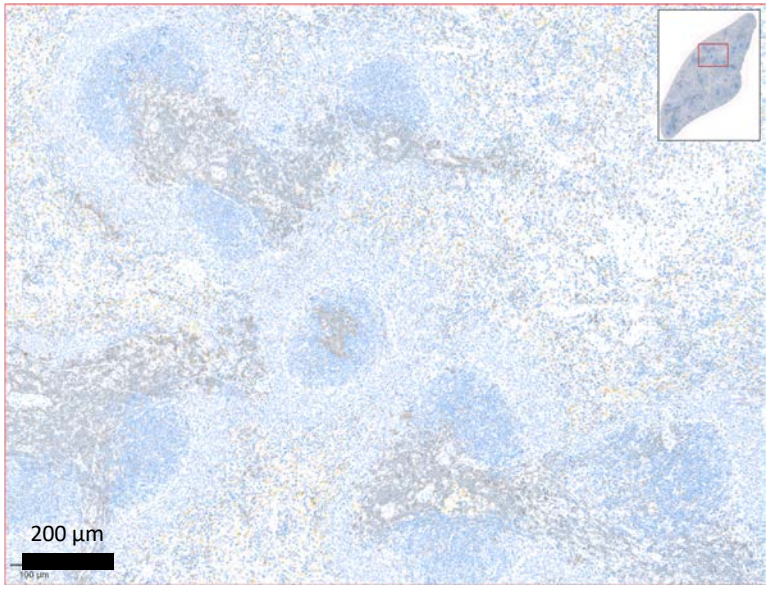


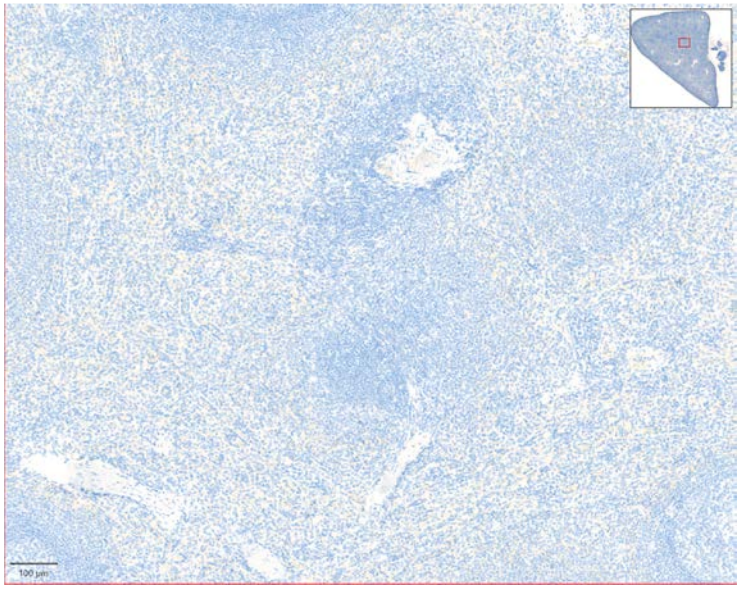
Coupes de rate de rat Fischer

Anti-CD3 polyclonal de référence
(1:200)

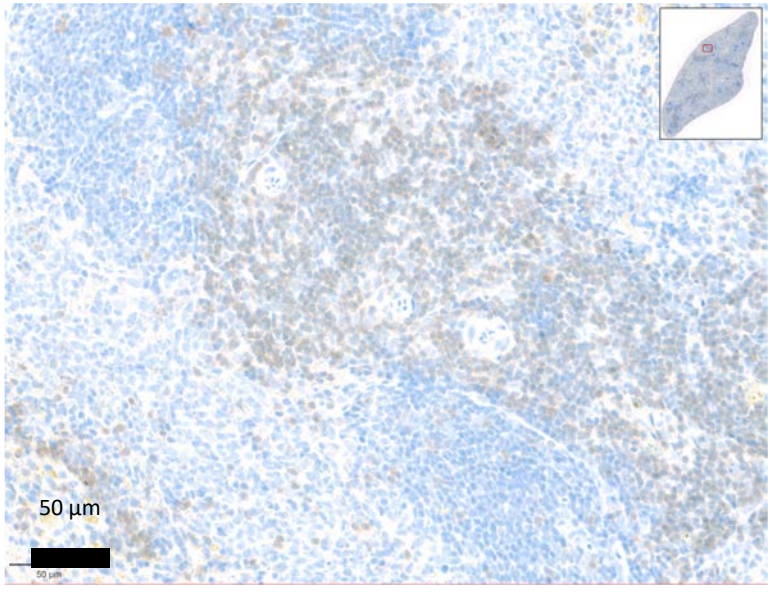
Anti-CD3 AD036 (ABCD)
Recombinant monoclonal
(1:100; CC2)

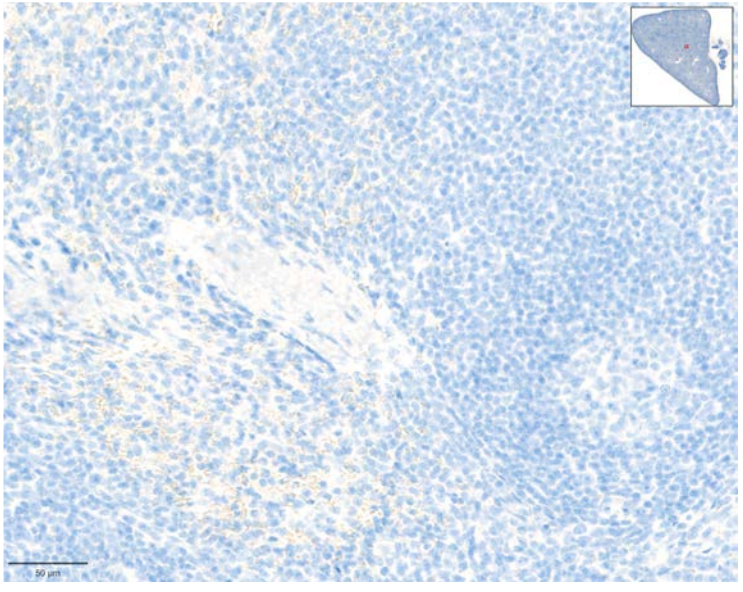
x 2.5





x 11.7



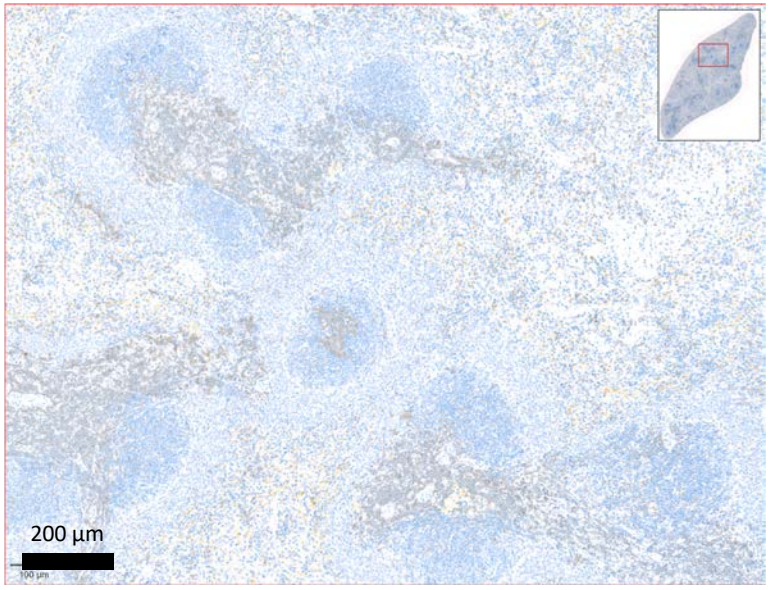


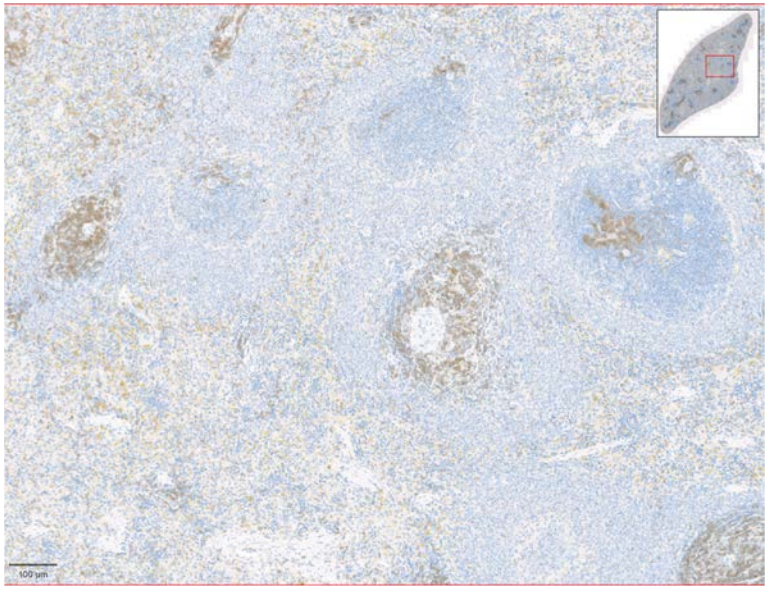
Coupes de rate de rat Fischer

Anti-CD3 polyclonal de référence
(1:200)

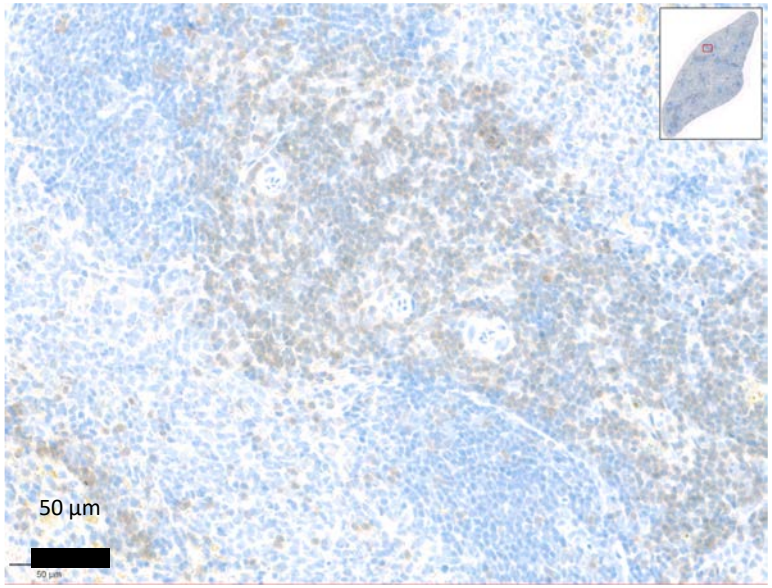
Anti-CD3 85061 (CST)
Recombinant monoclonal
(1:50; CC1)

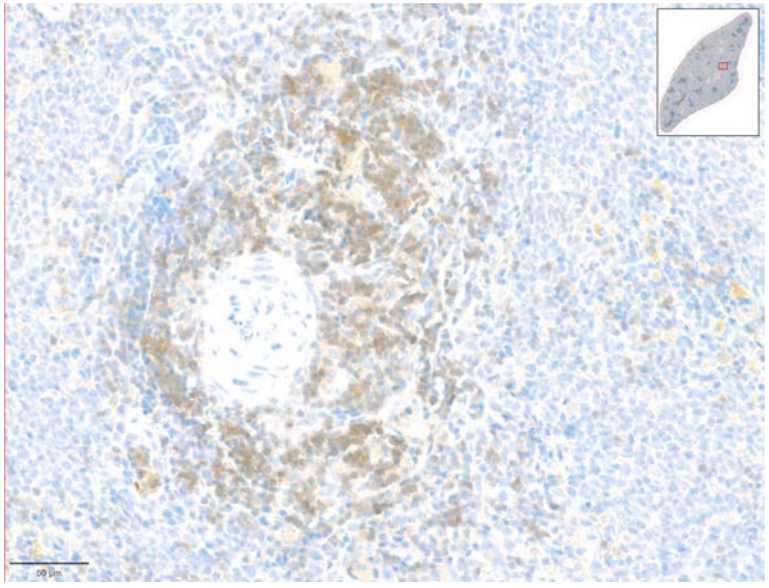
x 2.5





x 11.7



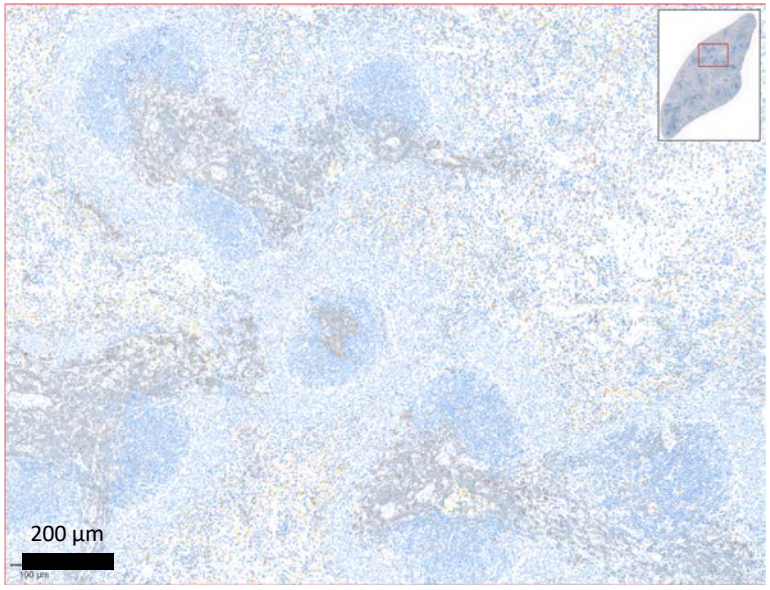


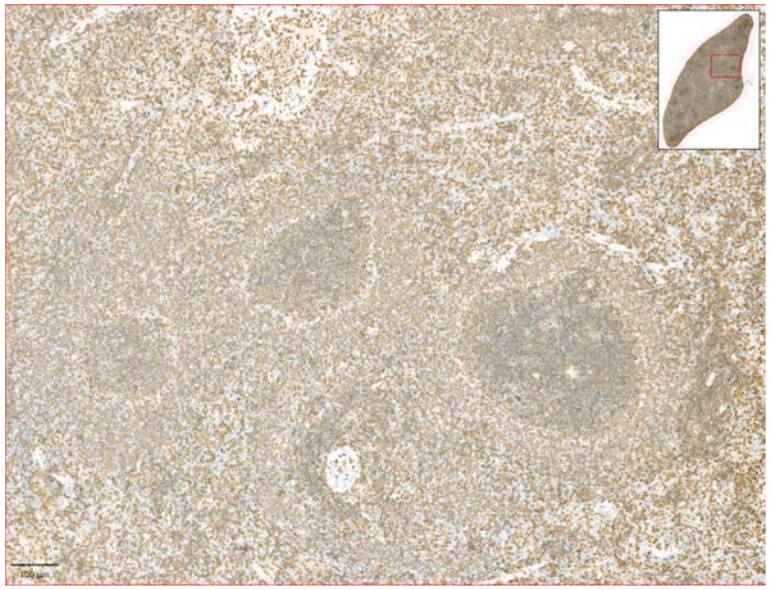
Coupes de rate de rat Fischer

Anti-CD3 polyclonal de référence
(1:200)

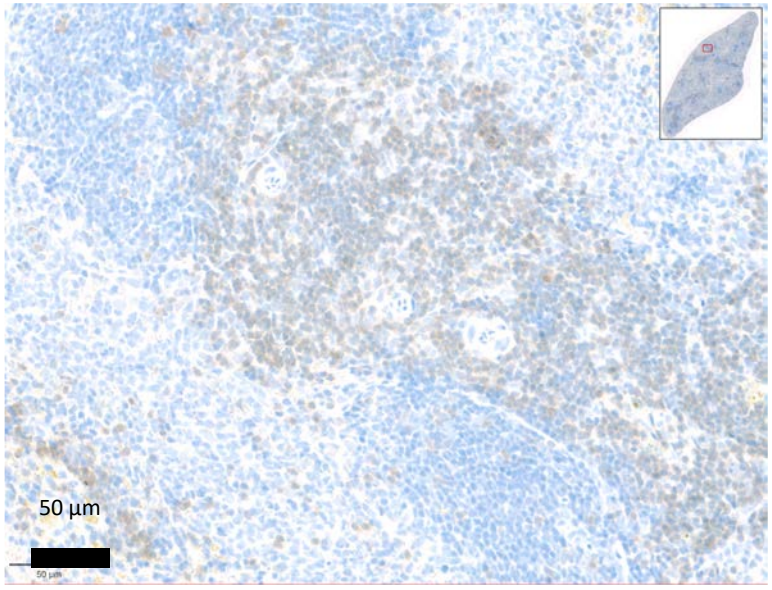
Anti-CD3 Yth12.5 (Absolute antibody)
Recombinant monoclonal
(1:100; CC2)

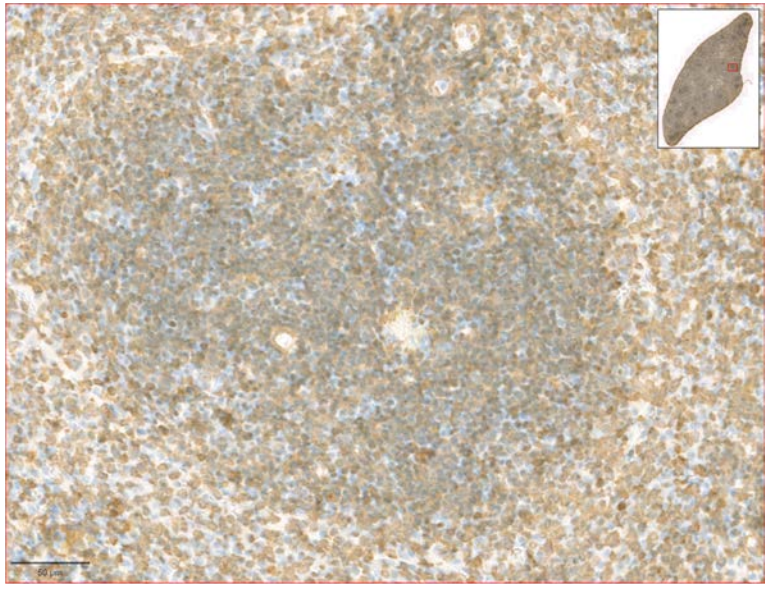
x 2.5





x 11.7



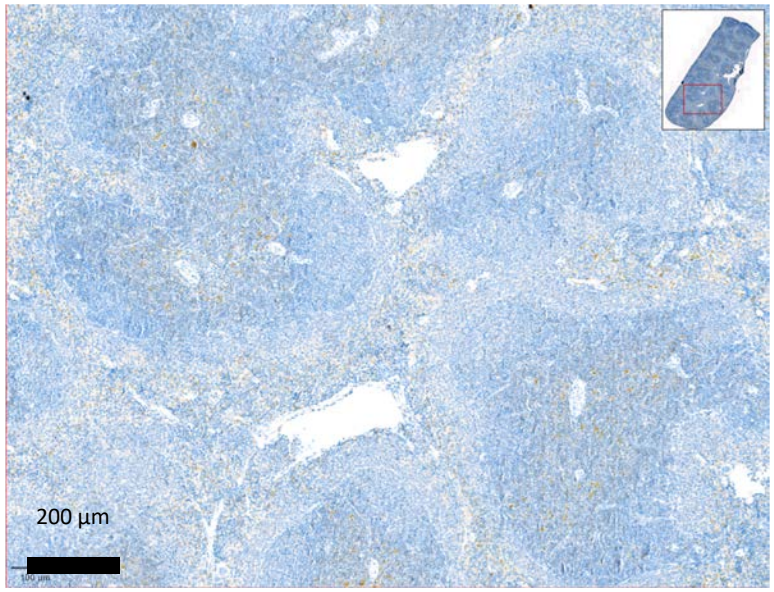


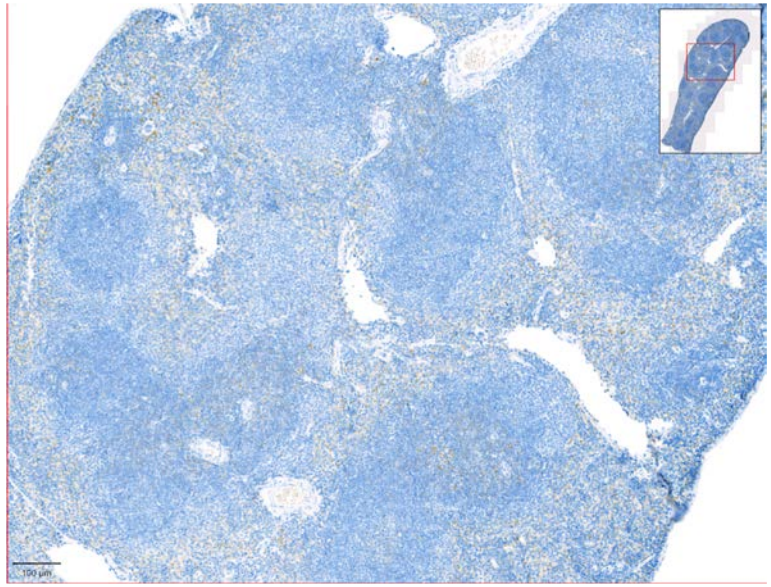
Coupes de rate de souris C57Bl6

Anti-CD3 polyclonal de référence
(1:2000)

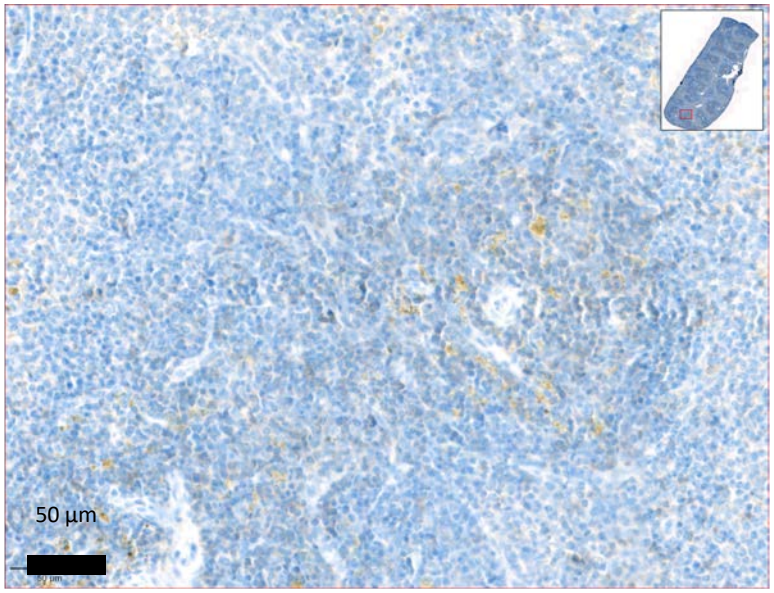
Anti-CD3 Ab16669** (Abcam)**
Recombinant monoclonal
(1:150; CC1)

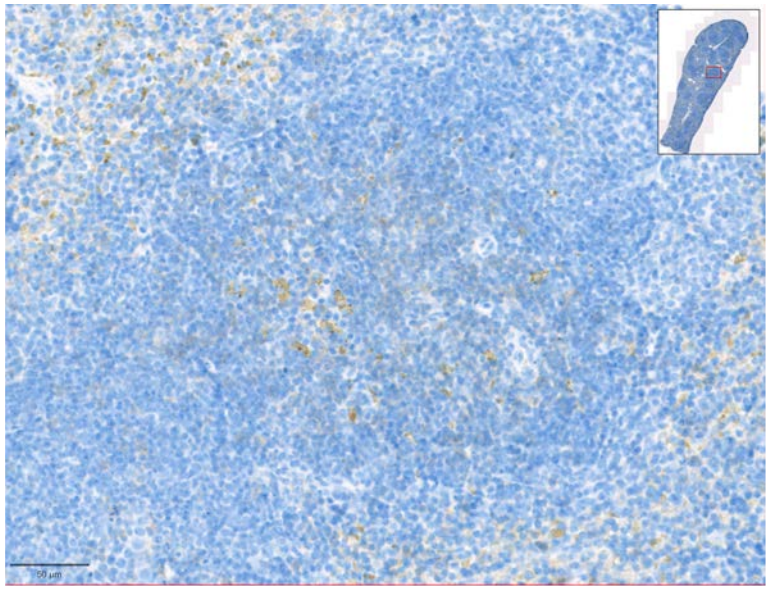
x 2.5





x 11.7



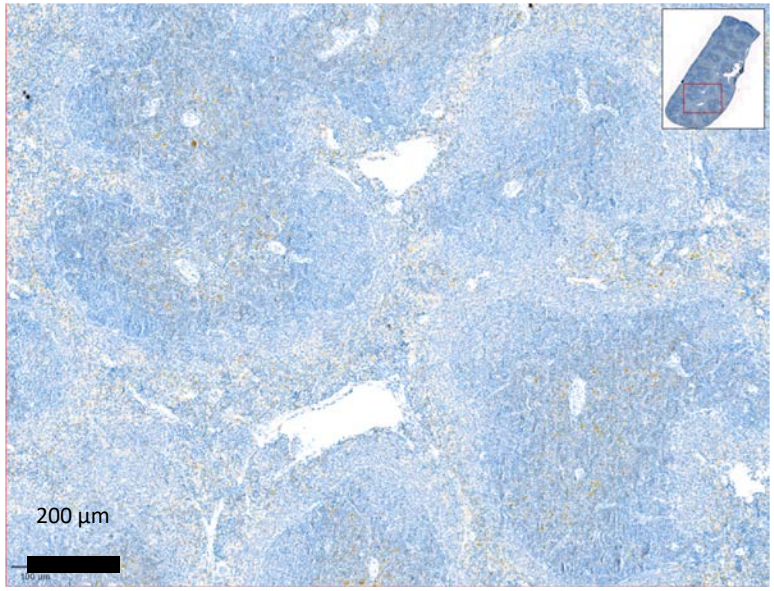


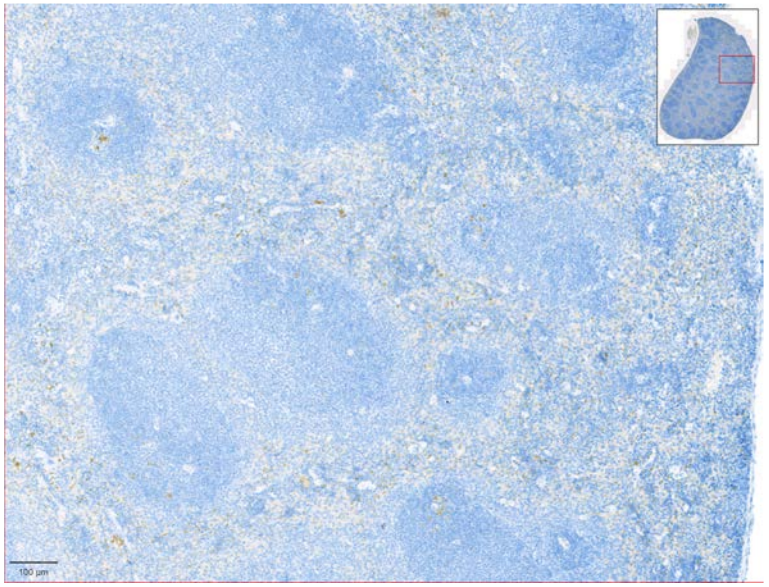
Coupes de rate de souris C57Bl6

Anti-CD3 polyclonal de référence
(1:2000)

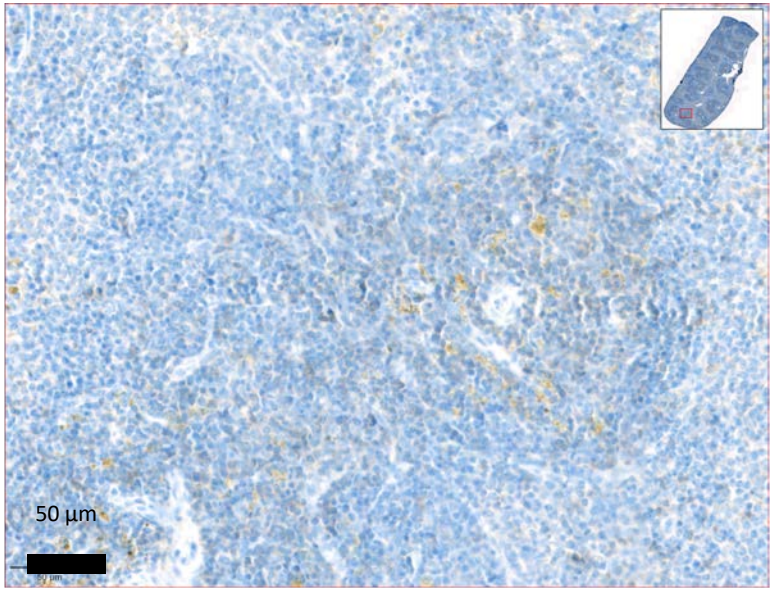
Anti-CD3 AD036 (ABCD)
Recombinant monoclonal
(1:100; CC2)

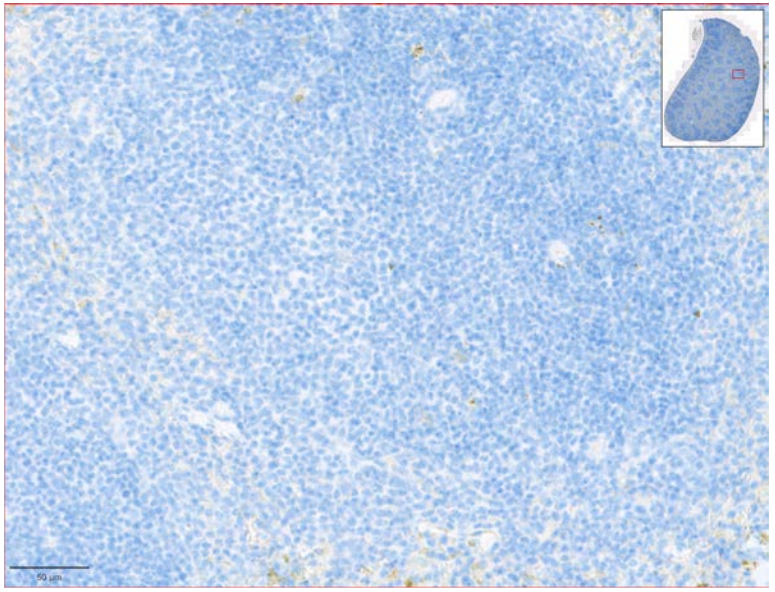
x 2.5





x 11.7



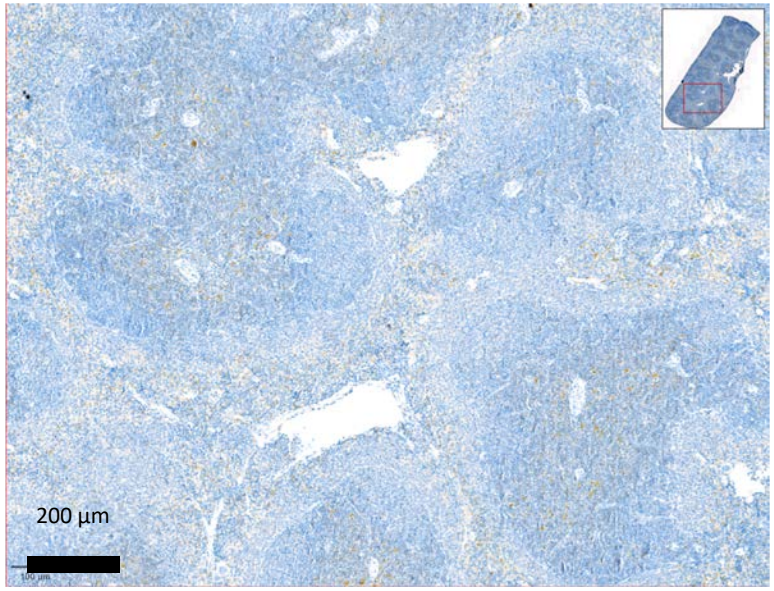


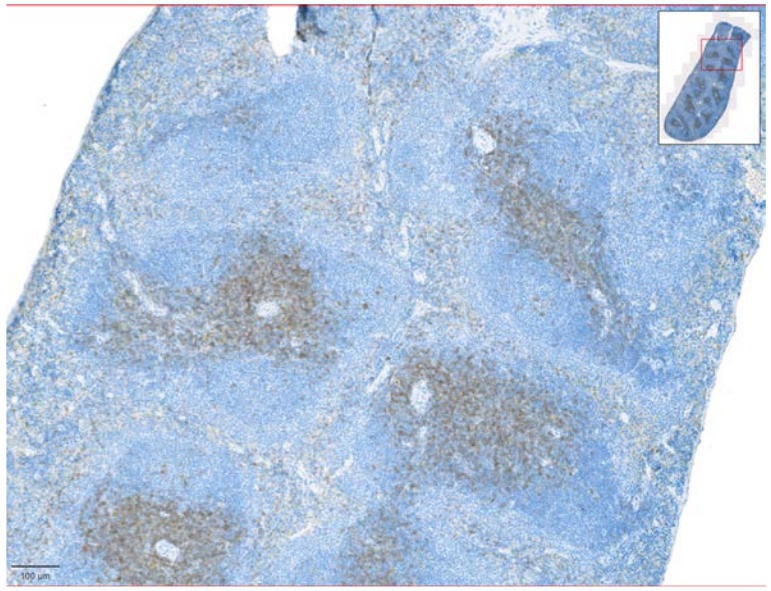
Coupes de rate de souris C57Bl6

Anti-CD3 polyclonal de référence
(1:2000)

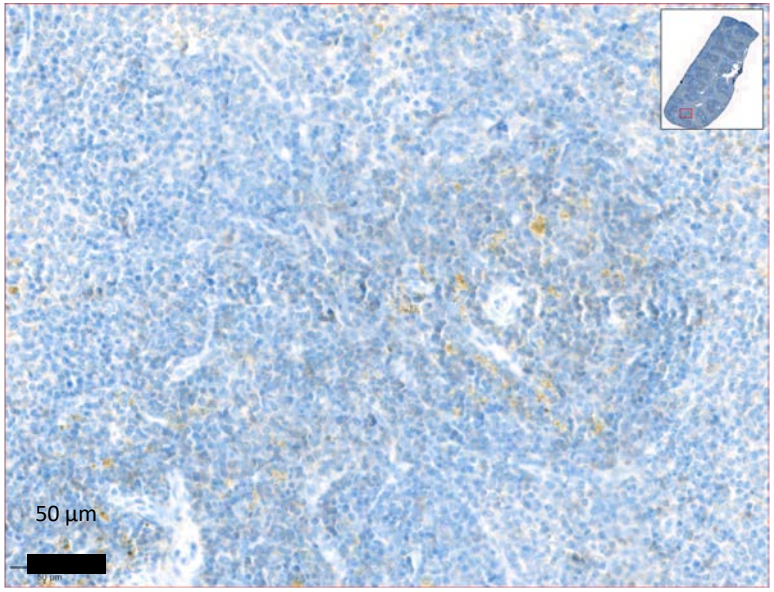
Anti-CD3 85061 (CST)
Recombinant monoclonal
(1:150; CC2 – *résultats similaires avec CC1*)

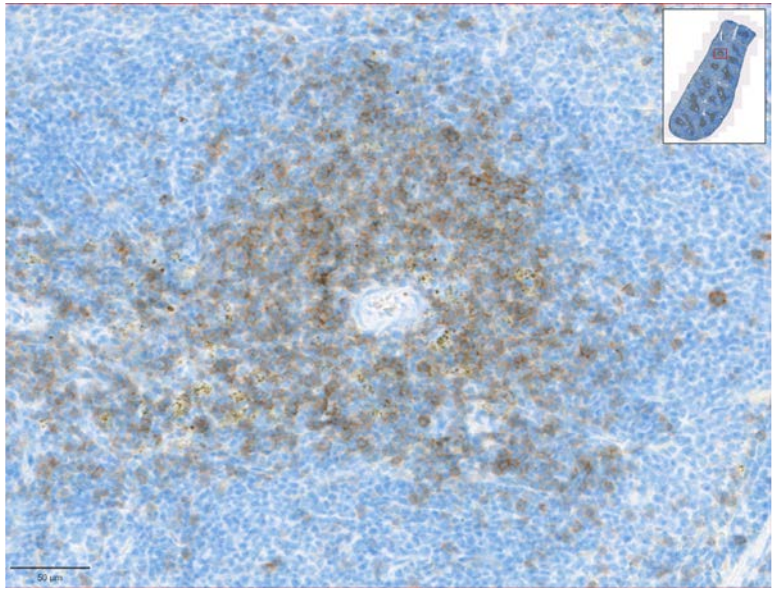
x 2.5





x 11.7



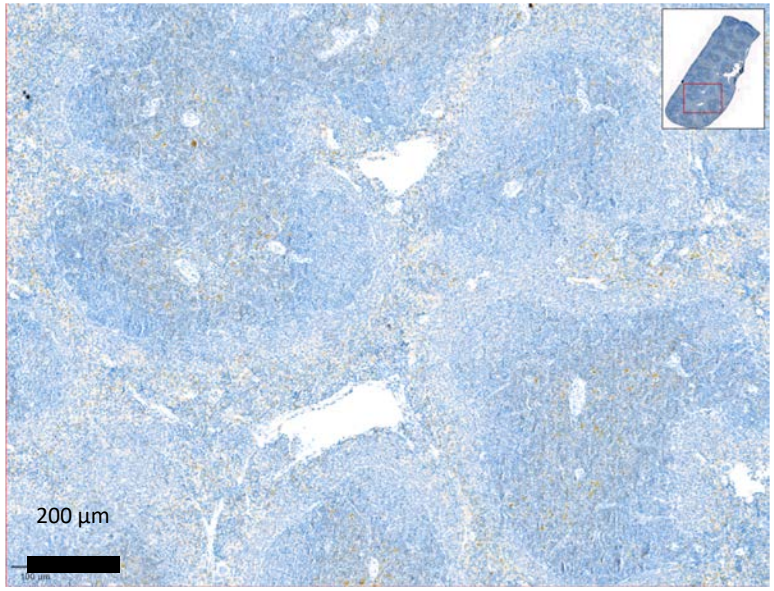


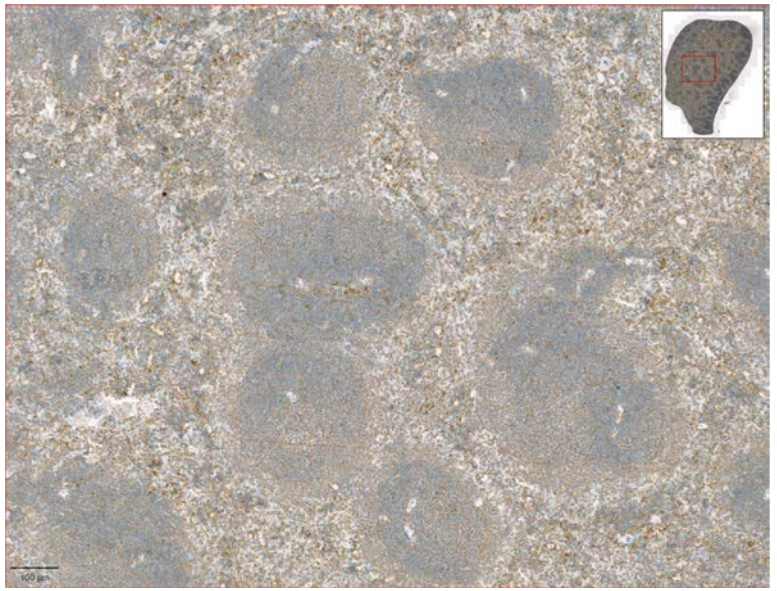
Coupes de rate de souris C57Bl6

Anti-CD3 polyclonal de référence
(1:2000)

Anti-CD3 Yth12.5 (Absolute antibody)
Recombinant monoclonal
(1:100; CC2)

x 2.5





x 11.7