

Materials and Methods

Experimental Strategy

The platform MICROPICELL evaluated four recombinant Fc Rabbit anti-CD4 antibodies on three formalin-fixed paraffin-embedded (FFPE) tissue sections from mouse (spleen, mammary gland, ganglion).

The first aim of this study was also to compare the results with those obtained with a CD4 antibody of animal origin used by the platform, which was not very specific, particularly on the mammary gland, and to know if the results obtained with these recombinant antibodies are better than the results obtained with an antibody of animal origin.

All tests were realized on the Discovery Ultra™ ROCHE®.

Various conditions were tested on these tissues for each antibody:

- Two conditions of antigen retrieval
- Three antibodies concentrations.

Antibody performance was assessed based on staining intensity, specificity, and background signal.

Biological Material

All formalin-fixed paraffin-embedded (FFPE) samples were already available on the platform. No animal has been sacrificed for these tests.

FFPE tissue sections were obtained from mouse (spleen, ganglion (MicroPicell) and mammary gland (CRCI2NA équipe 2)). Samples were collected and fixed 24h to 48h in neutral buffered formalin 10%, dehydrated, and embedded in paraffin. Paraffin-embedded tissue was cut into 3-µm-thick sections, mounted on slides, dried at room temperature overnight, then at 37°C 1h.

Antibody Testing

The tested rabbit monoclonal recombinant antibodies were:

- Anti-CD4 - ABCDantibodies - #ABCD_AD042 - Stock concentration: 120 µg/mL
- Anti-CD4 - [D7D2Z] – Cell Signaling - #25229 - Stock concentration: 155 µg/mL
- Anti-CD4 - [RM1013] - Abcam - #ab288724 - Stock concentration: 525 µg/mL
- Anti-CD4 - [250263G12] - Proteintech - #86-300-3RR - Stock concentration: 1000 µg/mL

Immunohistochemistry was performed on a Discovery Ultra™ ROCHE® automated staining instrument, using ROCHE® reagents, according to the manufacturer's instructions.

The recombinant anti-CD4 antibodies were tested under these conditions:

Slides were de-paraffinized with Discovery Wash (*Roche*, #07311079001). Then epitope retrieval was performed with:

1. **CC1 (pH 8)** (*Roche*, #05279801001): Heat-induced epitope retrieval (HIER) at 95°C for 40 min,
2. **CC2 (pH 6)** (*Roche*, #05279798001): HIER at 95°C for 40 min.

Endogenous peroxidase was blocked with Discovery Inhibitor CM from the Chromomap DAB kit (*Roche*, #05266645001) for 8 min.

The antibodies were diluted in Antibody Diluent (*Roche*, #05261899001) with the ABCDantibodies, Proteintech, and Abcam CD4 antibodies or SignalStain® Antibody Diluent (*Ozyme*, #8112S) with the Cell Signaling CD4 antibody.

The antibodies dilutions used were those recommended by the antibodies data sheets.

* Anti-CD4 - ABCDantibodies - #ABCD_AD042: 1/50 (2.4 µg/mL), 1/100 (1.2 µg/mL), 1/200 (0.6 µg/mL)

* Anti-CD4 - [D7D2Z] – Cell Signaling - #25229: 1/50 (3.1 µg/mL), 1/100 (1.55 µg/mL), 1/200 (0.775 µg/mL)

* Anti-CD4 - [RM1013] - Abcam - #ab288724: 1/500 (1.05 µg/mL), 1/1000 (0.525 µg/mL), 1/2000 (0.262 µg/mL)

* Anti-CD4 - [250263G12] - Proteintech - #86-300-3RR: 1/500 (2 µg/mL), 1/1000 (1 µg/mL), 1/2000 (0.5 µg/mL).

All antibodies were incubated for 60 min at room temperature.

Signal was enhanced using the OmniMap anti-rabbit detection kit (*Roche*, #05269679001) for 16 min, according to the manufacturer's instructions.

Slides were incubated with DAB from the Chromomap DAB kit (*Roche*, #05266645001) then counterstained with hematoxylin II (*Roche*, #05277965001) for 8 min, followed by Bluing reagent (*Roche*, #05266769001) for 4 min.

Slides were then dehydrated and coverslipped with Expert Mounting Medium (*CellPath*, #SEA-1604-00A).

In parallel, negative controls were realized for each tissue (spleen, mammary gland, ganglion), by replacing the antibody by the antibody diluent alone.

Image Acquisition

Images were acquired using a scanner S360™ Hamamatsu®; using the 20X objective.

Results Summary

Two recombinant antibodies (Abcam - #ab288724 and Proteintech - #86-300-3RR) were validated in all tissues (spleen, mammary gland, ganglion). The staining conditions are indicated below.

The other antibodies (ABCDantibodies - #ABCD_AD042, Cell Signaling - #25229) did not produce specific staining under tested conditions, but conditions may be optimized to obtain best results.

Any staining was observed on the negative controls.

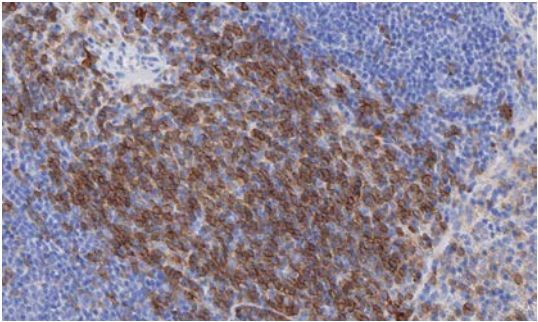
Supplier Reference	Clône	Epitope retrieval	Dilution	Amplification
Abcam #ab288724	RM1013	CC1 – 40 min – 95°C	1/500	OmniMap Rabbit HRP
Proteintech - #86-300-3RR	250263G12	CC1 – 40 min – 95°C	1/2000 (may be more diluted)	OmniMap Rabbit HRP

Recombinant antibodies significantly reduced the per-slide cost (between 0.22€ and 0.76 €/slide) compared to the reference antibody of animal origin (8.55€/slide), representing up to a really cost reduction.

MOUSE
SPLEEN

Recombinant monoclonal antibody
Anti-CD4 ABCD antibodies #ABCD_AD042

Reference antibody
CD4 4SM95
Invitrogen #14-9766-82
Staining +



CD4 1/50

(2,4 µg/mL) 1h RT, Omnimap anti-rabbit HRP 16min, DAB

CD4 1/100

(1,2 µg/mL) 1h RT, Omnimap anti-rabbit HRP 16min, DAB

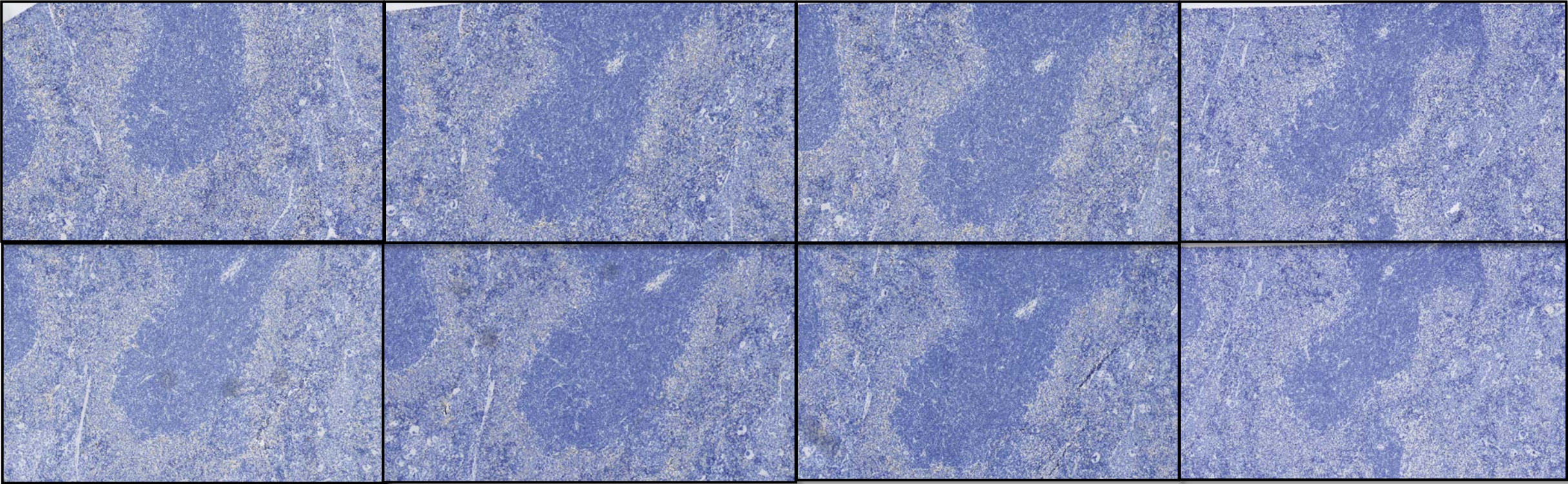
CD4 1/200

(0,6 µg/mL) 1h RT, Omnimap anti-rabbit HRP 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



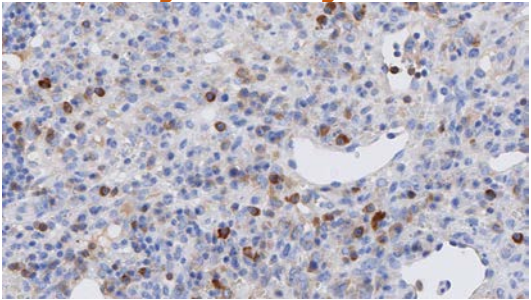
Staining -

Clean negative controls

MOUSE
MAMMARY
TUMOR

Recombinant monoclonal antibody
Anti-CD4 ABCD antibodies #ABCD_AD042

Reference antibody
CD4 4SM95
Invitrogen # 14-9766-82
Staining + but background



CD4 1/50

(2,4 µg/mL) 1h RT, Omnimap anti-rabbit HRP 16min, DAB

CD4 1/100

(1,2 µg/mL) 1h RT, Omnimap anti-rabbit HRP 16min, DAB

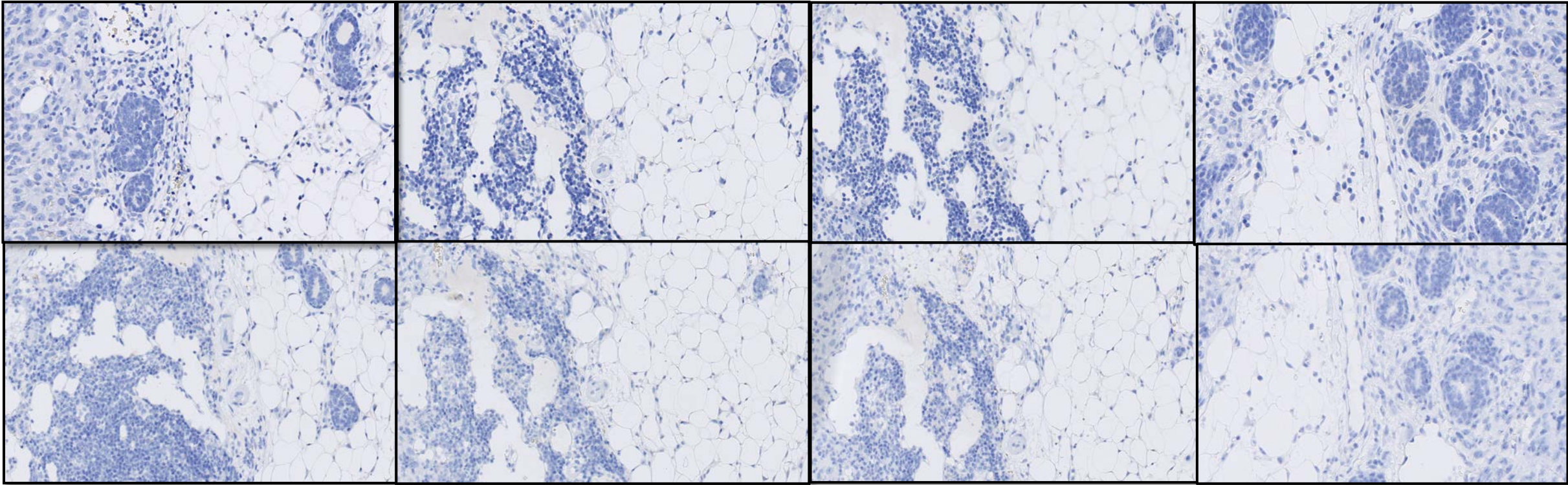
CD4 1/200

(0,6 µg/mL) 1h RT, Omnimap anti-rabbit HRP 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



Staining -

Clean negative controls

MOUSE
GANGLION

Recombinant monoclonal antibody
Anti-CD4 ABCD antibodies #ABCD_AD042

CD4 1/50

(2,4 µg/mL) 1h RT, Omnimap anti-
rabbit HRP 16min, DAB

CD4 1/100

(1,2 µg/mL) 1h RT, Omnimap anti-
rabbit HRP 16min, DAB

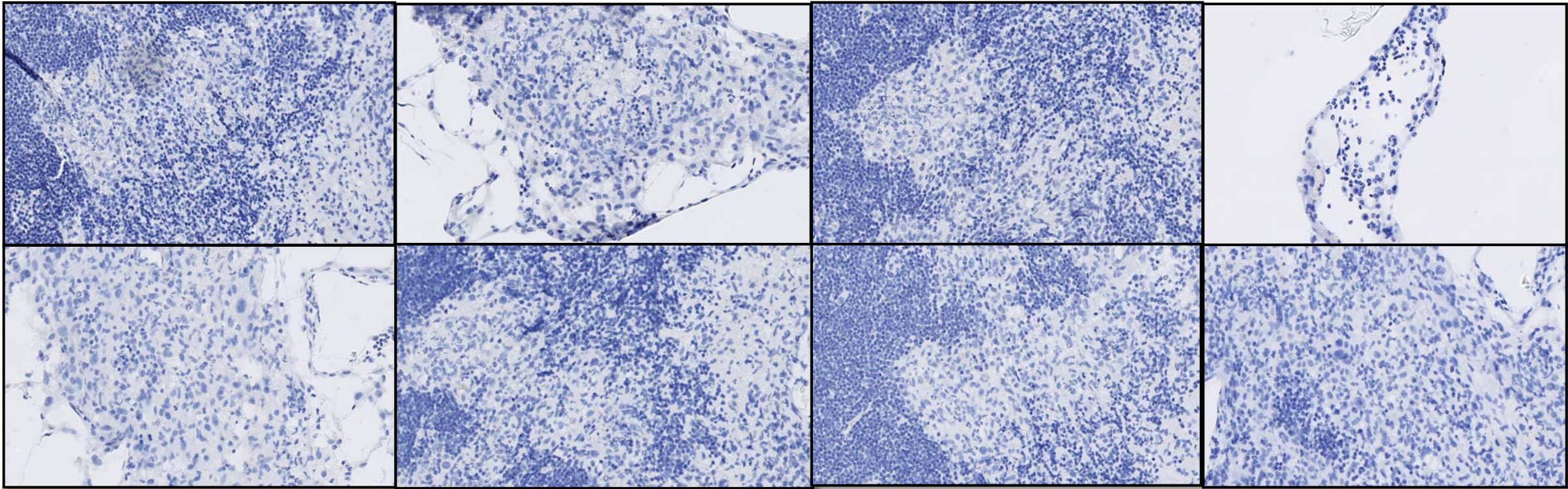
CD4 1/200

(0,6 µg/mL) 1h RT, Omnimap anti-
rabbit HRP 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



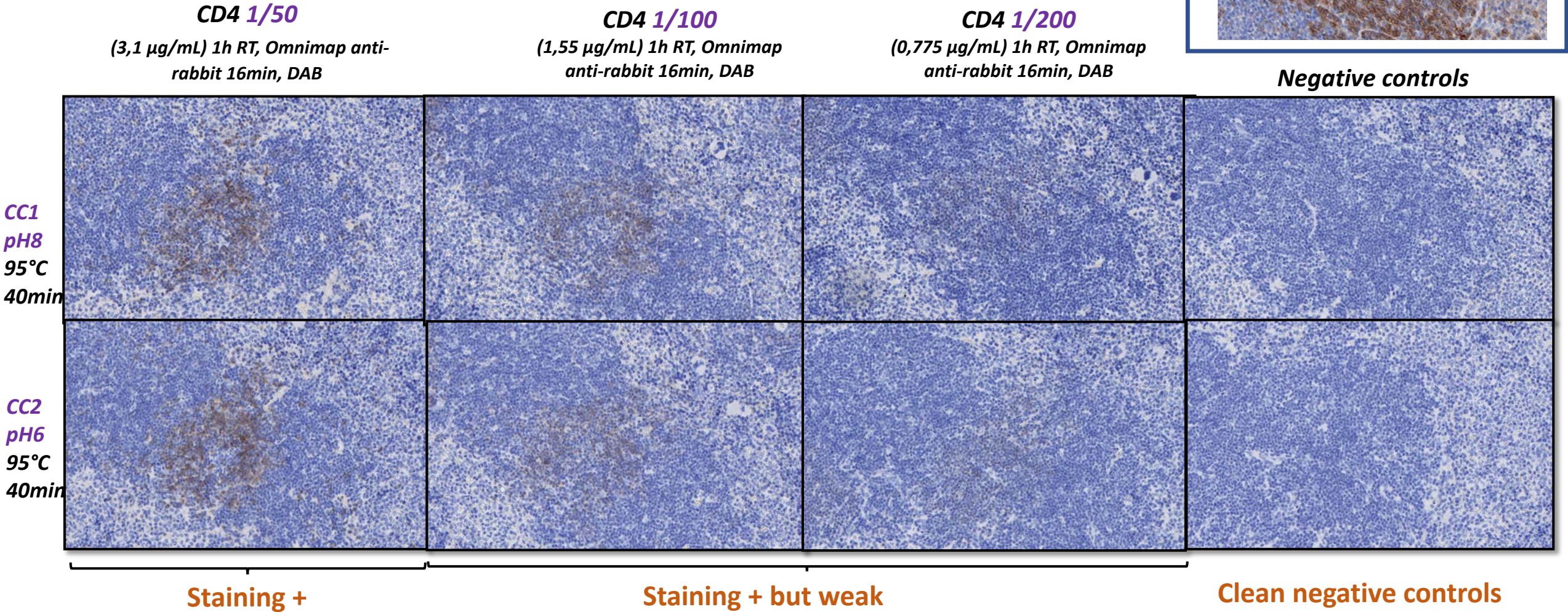
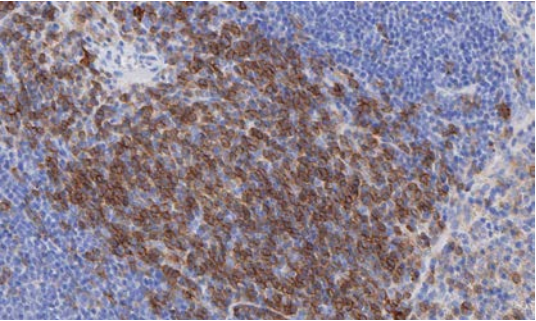
Staining -

Clean negative controls

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SPLEEN

Recombinant monoclonal antibody
Anti-CD4 Cell Signaling #25229

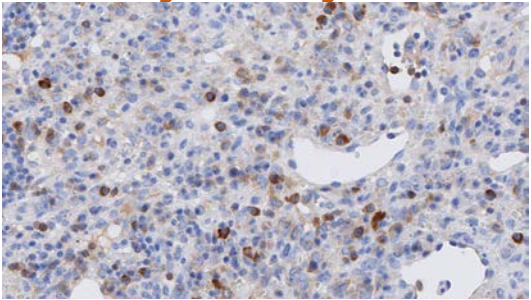
Reference antibody
CD4 4SM95
Invitrogen ref 14-9766-82
Staining +



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TUMOR

Recombinant monoclonal antibody
Anti-CD4 Cell Signaling #25229

Reference antibody
CD4 4SM95
Invitrogen ref 14-9766-82
Staining + but background



CD4 1/50

(3,1 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

CD4 1/100

(1,55 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

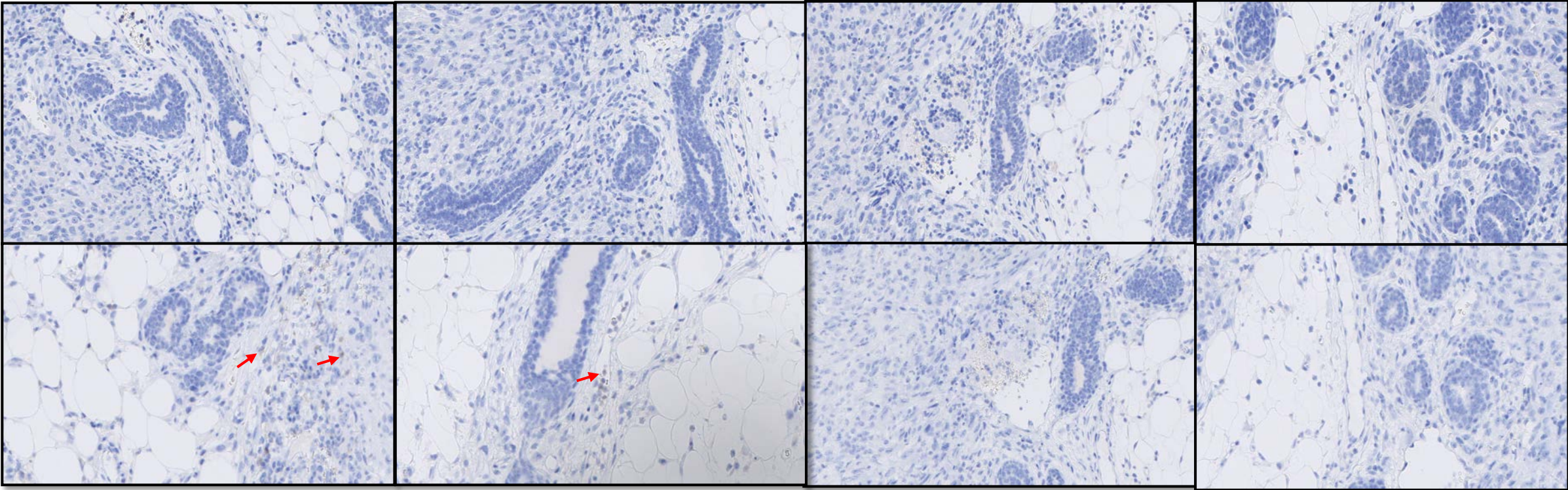
CD4 1/200

(0,775 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



Staining + but weak

Staining -

Clean negative controls

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Recombinant monoclonal antibody
Anti-CD4 Cell Signaling #25229

CD4 1/50

(3,1 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

CD4 1/100

(1,55 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

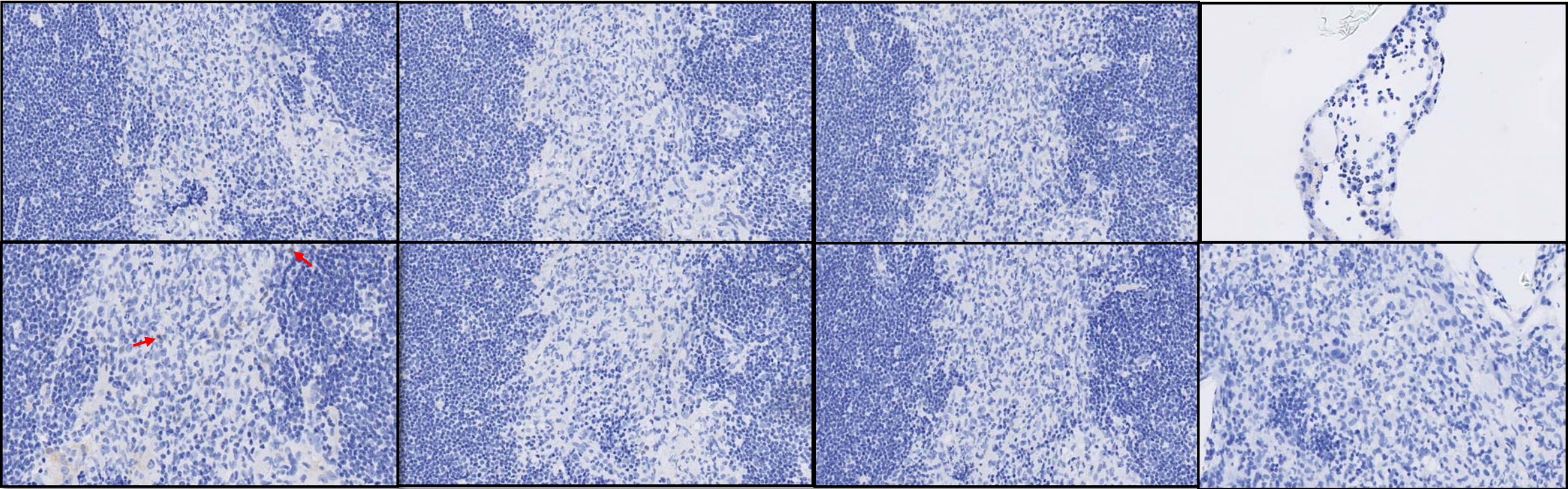
CD4 1/200

(0,775 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



Staining + (Few isolated cells)

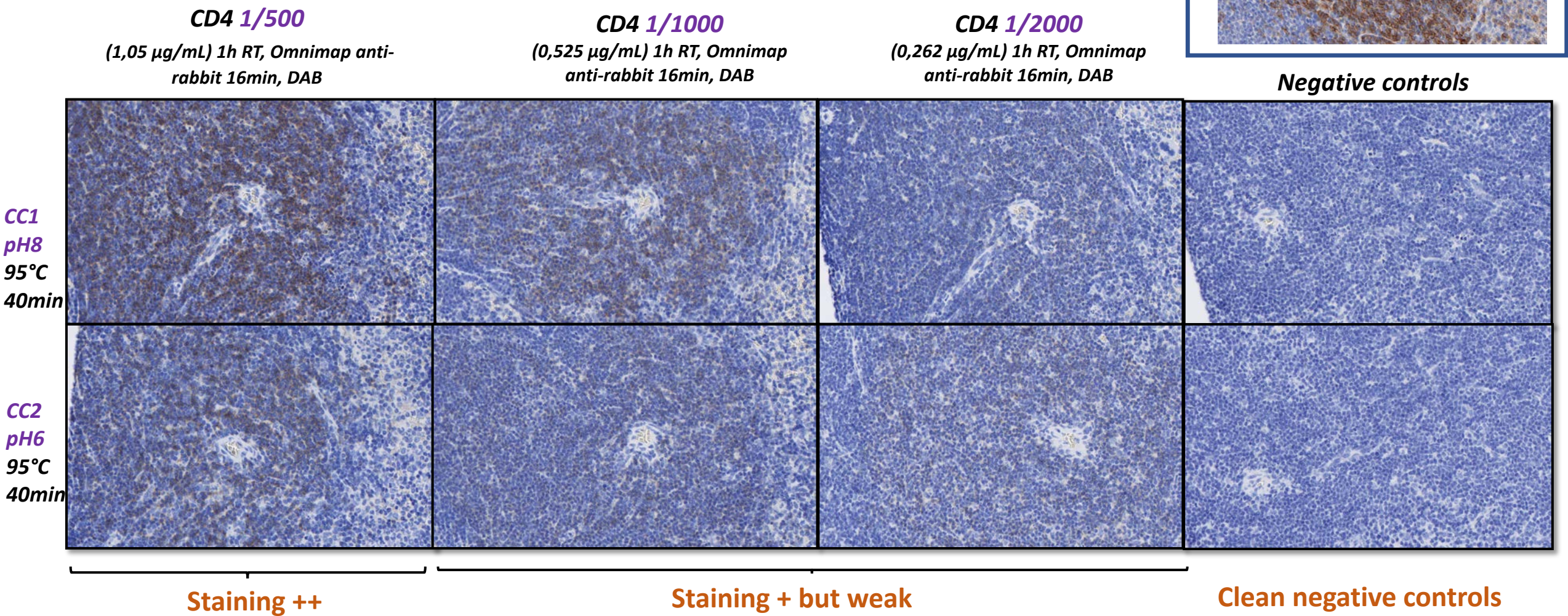
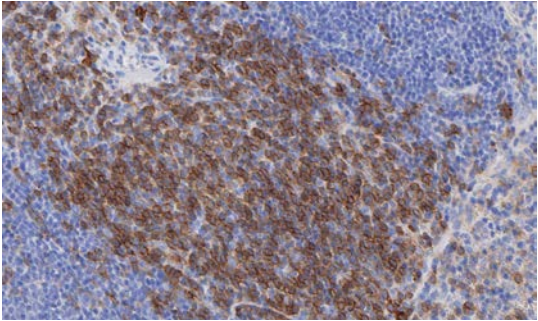
Staining -

Clean negative controls

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SPLEEN

Recombinant monoclonal antibody **Anti-CD4 Abcam # ab288724**

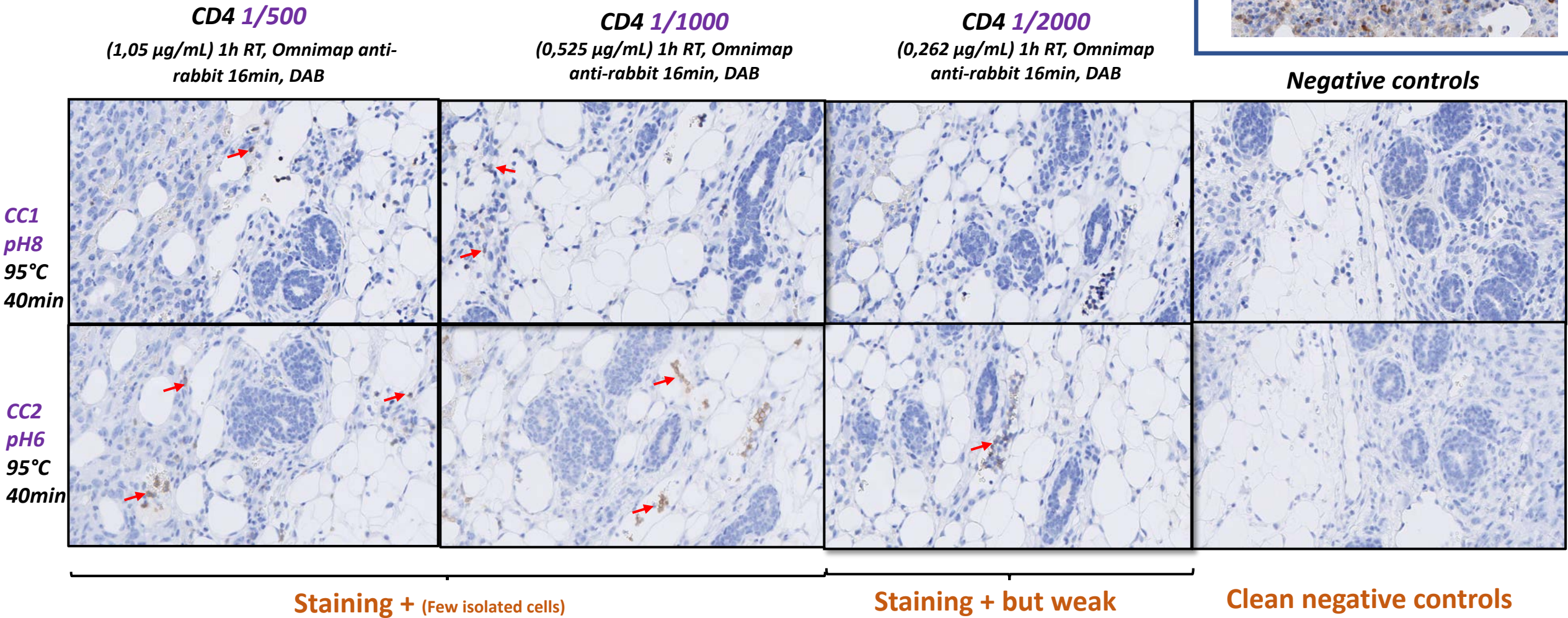
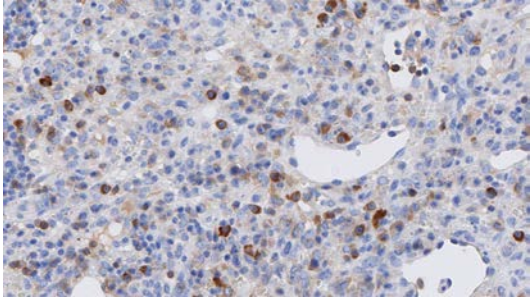
Reference antibody
CD4 4SM95
Invitrogen ref 14-9766-82
Staining +



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TUMOR

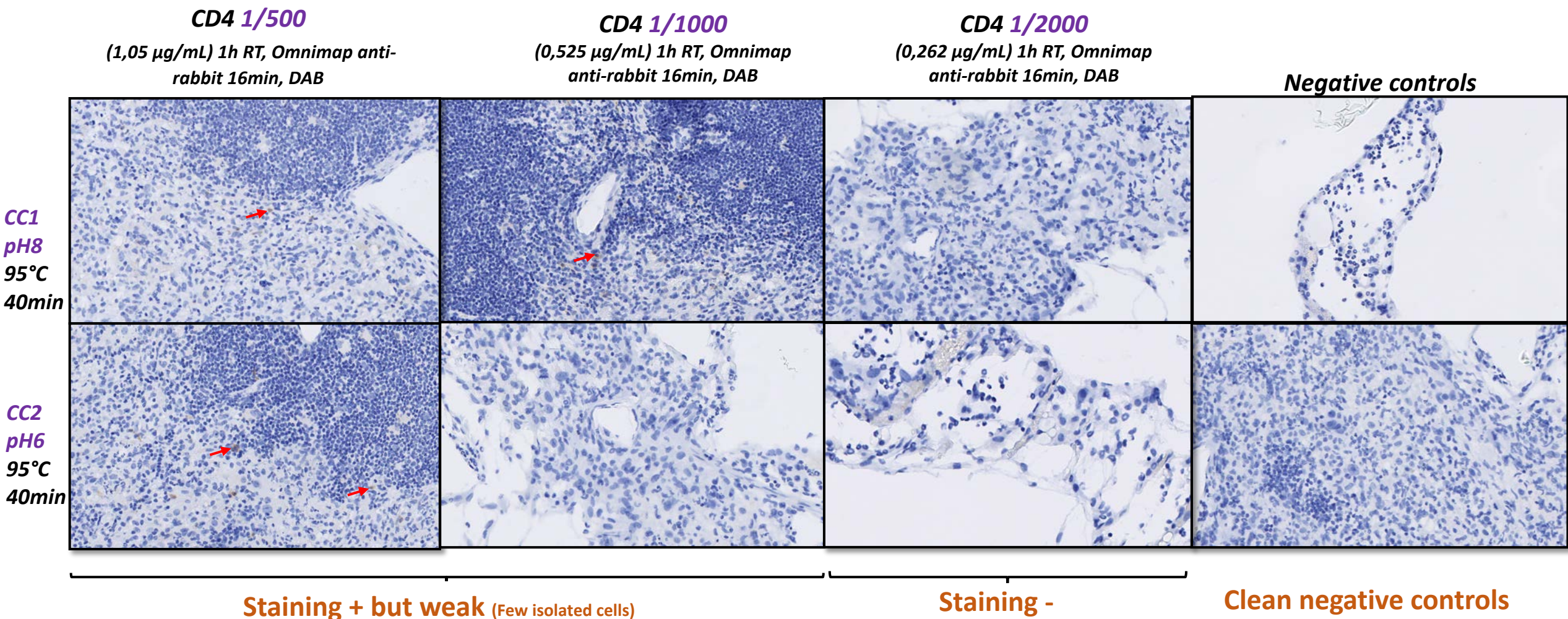
Recombinant monoclonal antibody **Anti-CD4 Abcam # ab288724**

Reference antibody
CD4 4SM95
Invitrogen ref 14-9766-82
Staining + but background



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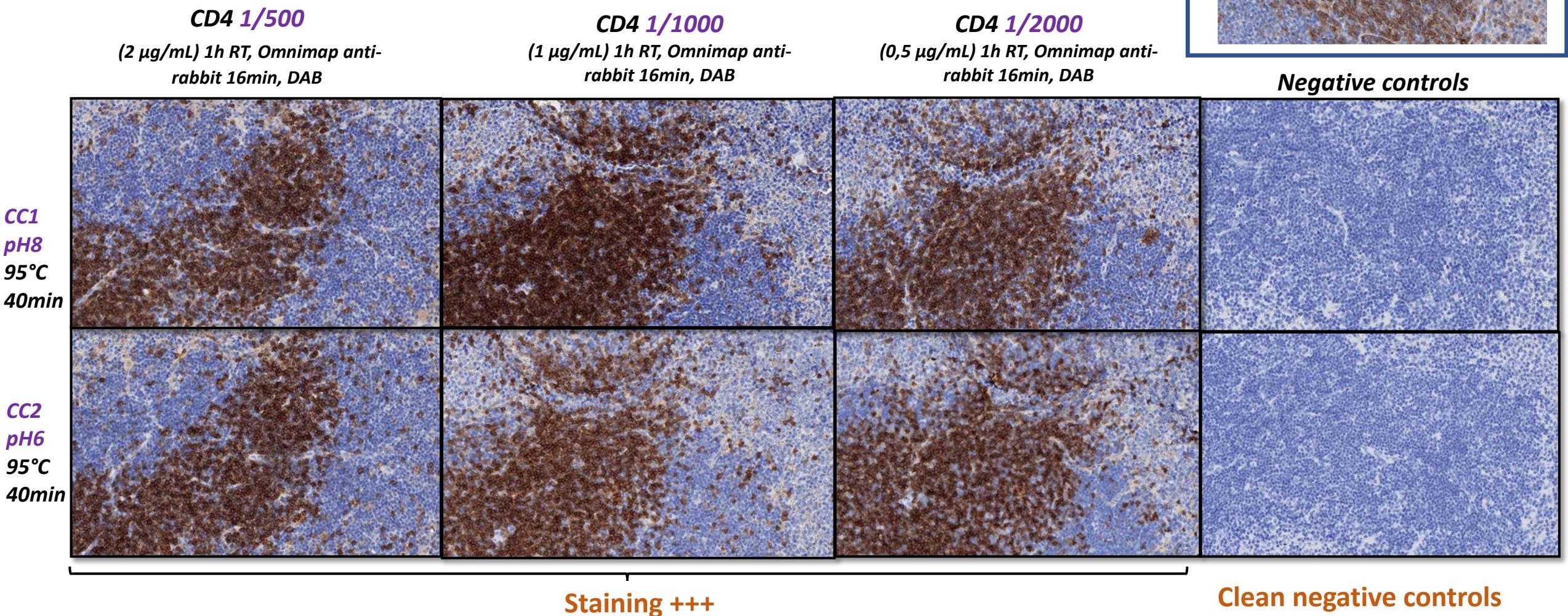
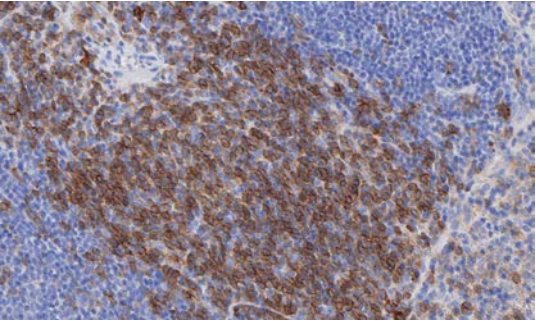
Recombinant monoclonal antibody **Anti-CD4 Abcam # ab288724**



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Recombinant monoclonal antibody **Anti-CD4 Proteintech #86300-3-RR**

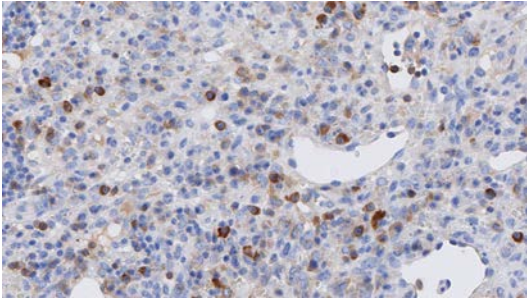
Reference antibody
CD4 4SM95
Invitrogen ref 14-9766-82
Staining +



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TUMOR

Recombinant monoclonal antibody **Anti-CD4 Proteintech #86300-3-RR**

Reference antibody
CD4 4SM95
Invitrogen ref 14-9766-82
Staining + but background



CD4 1/500

(2 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

CD4 1/1000

(1 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

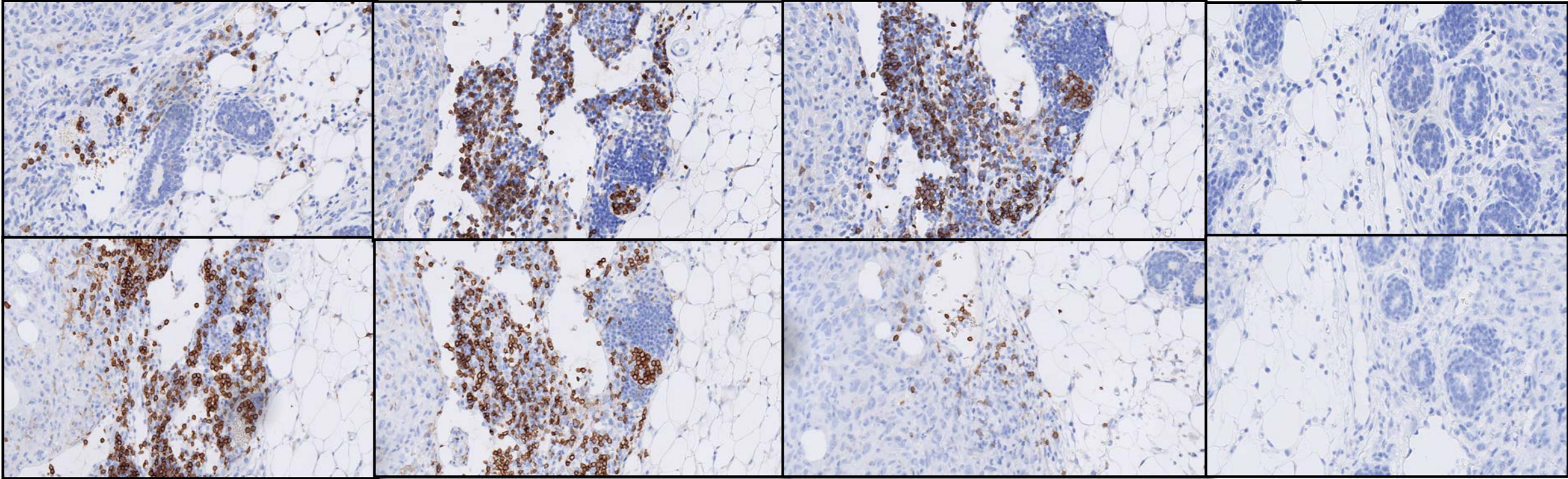
CD4 1/2000

(0,5 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



Staining +++

Clean negative controls

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Recombinant monoclonal antibody **Anti-CD4 Proteintech #86300-3-RR**

CD4 1/500

(2 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

CD4 1/1000

(1 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

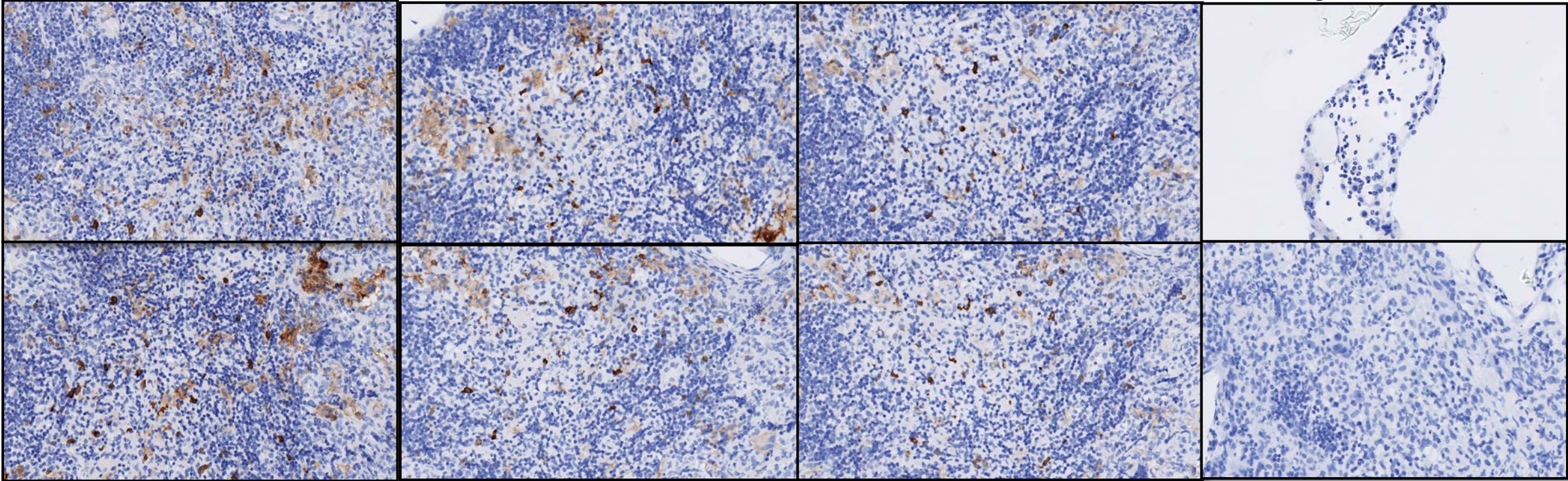
CD4 1/2000

(0,5 µg/mL) 1h RT, Omnimap anti-rabbit 16min, DAB

Negative controls

CC1
pH8
95°C
40min

CC2
pH6
95°C
40min



Staining +++ but background

Clean negative controls