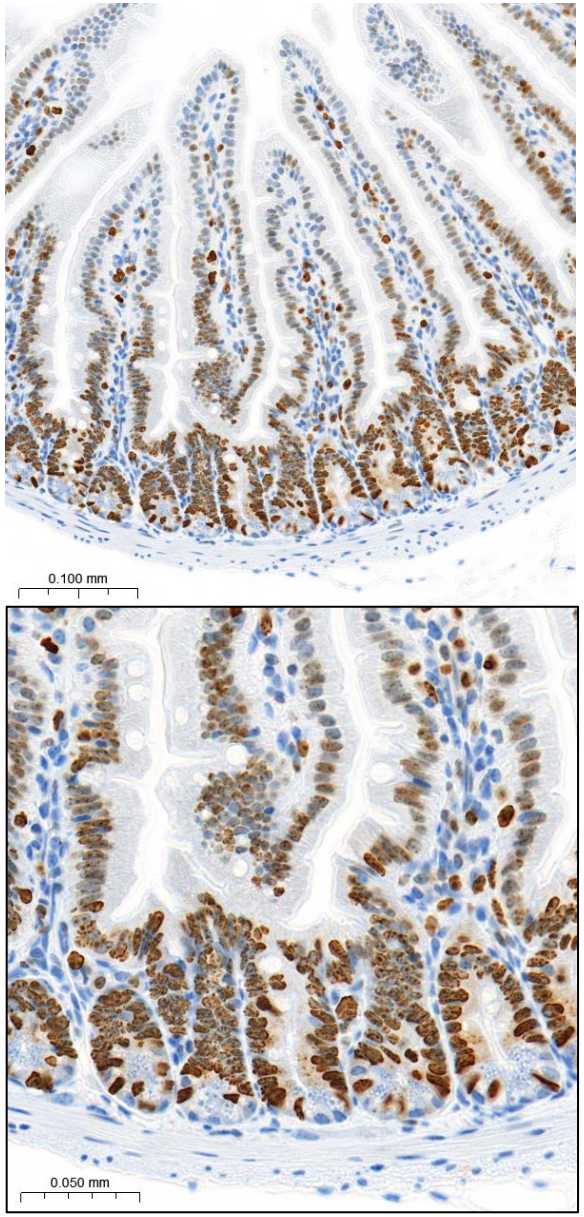
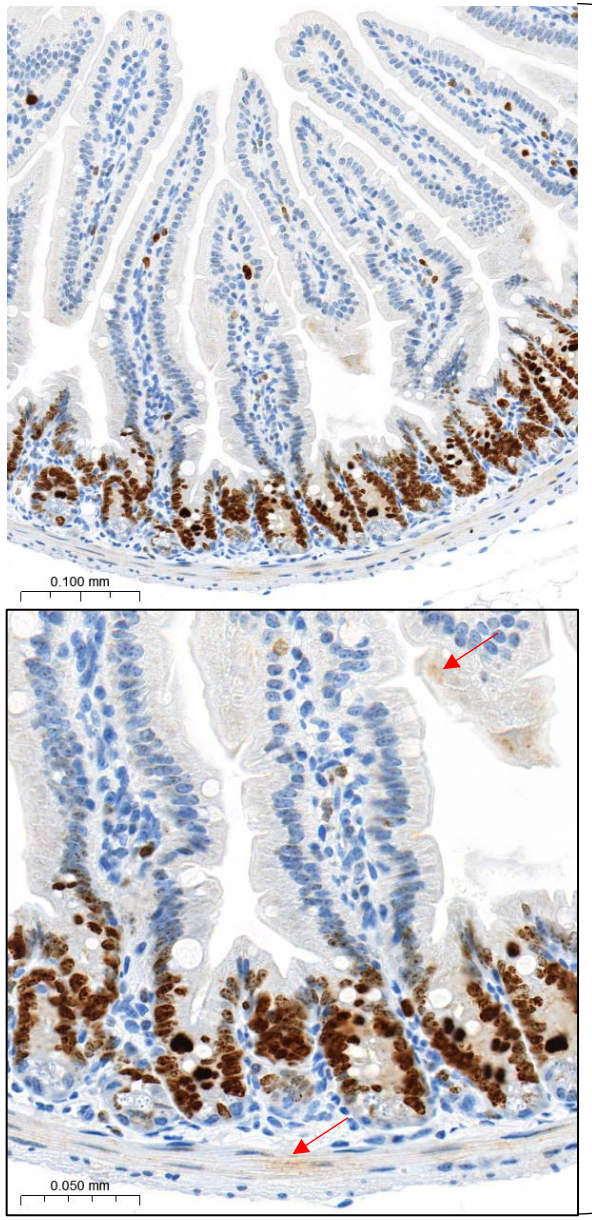
Mouse sample (intestine)

Reference monoclonal anti-Ki67
1/250 ST



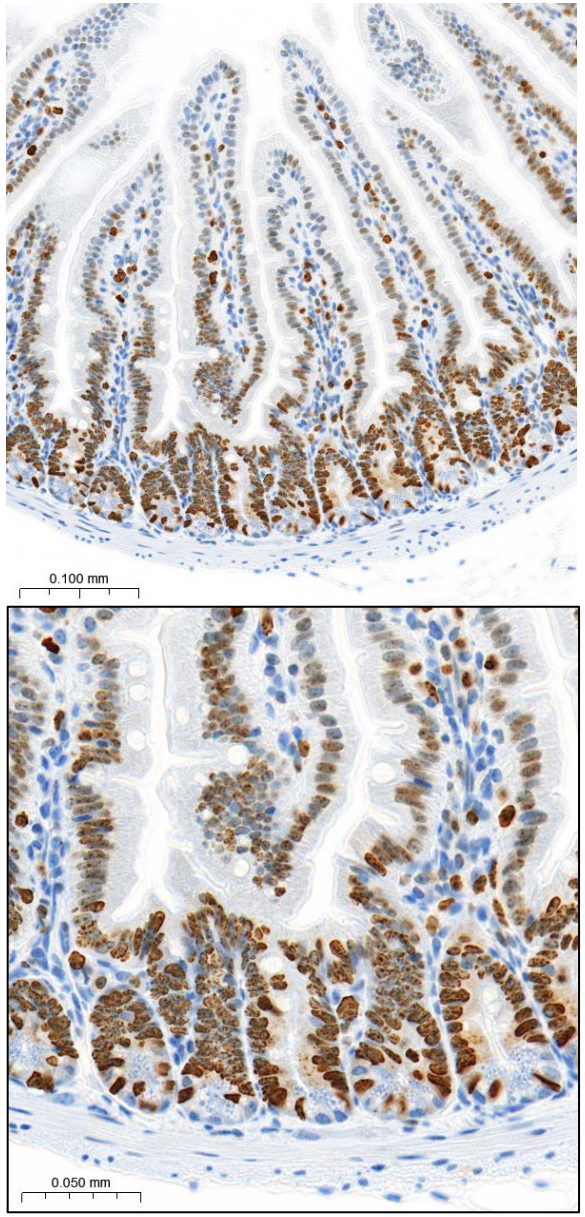
Recombinant monoclonal
anti-Ki67 Invitrogen MA5-14520
1/250 ST



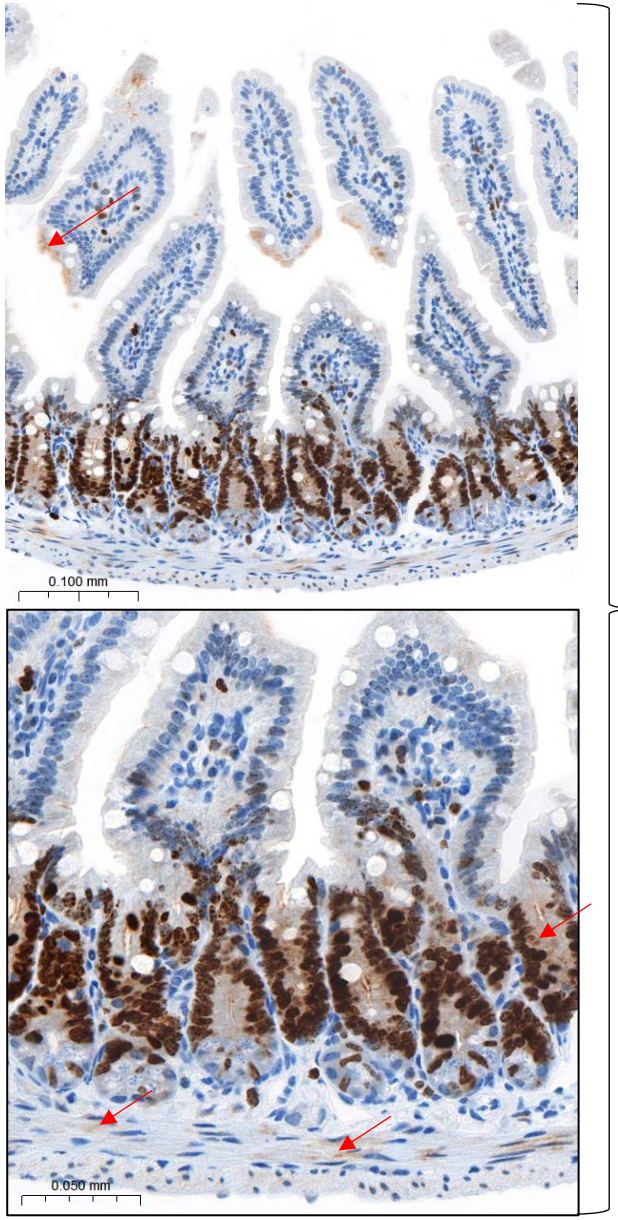
Signal more intense and less extensive than with the reference antibody. Presence of some background noise:

Mouse sample (intestine)

Reference monoclonal anti-Ki67
1/250 ST



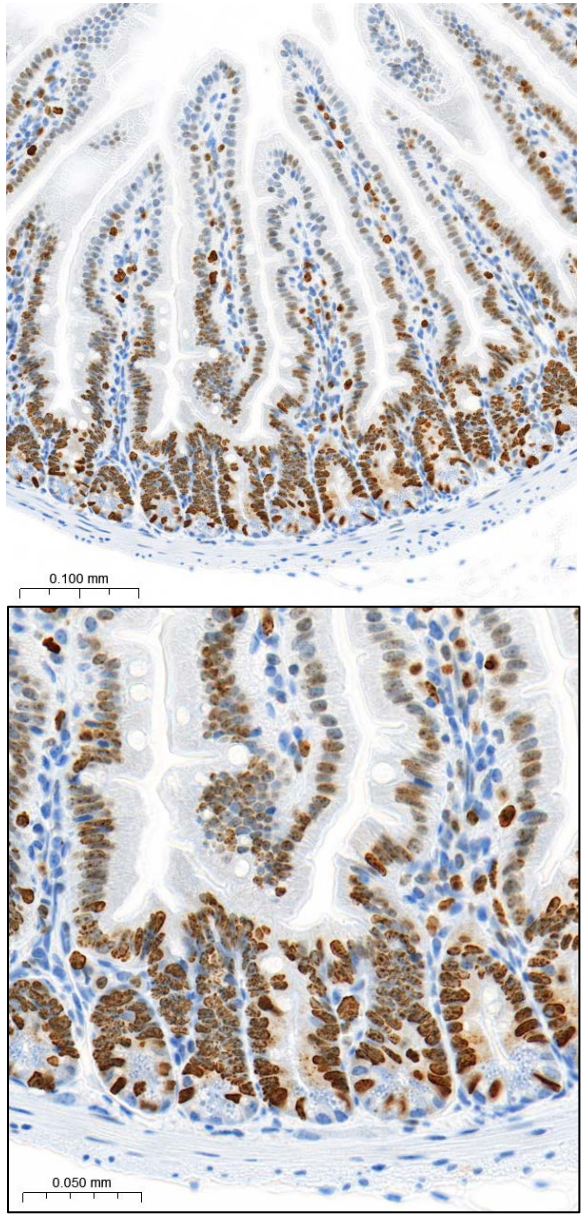
Recombinant monoclonal
anti-Ki67 Abcam Ab16667
1/100 ST



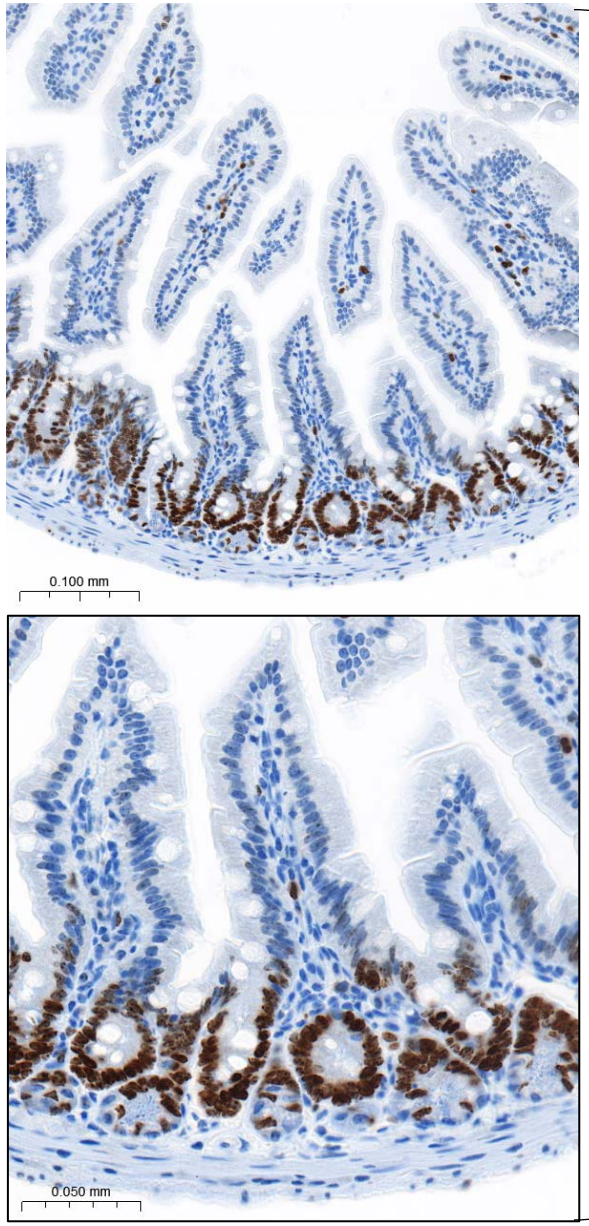
Signal more intense and less extensive than with the reference antibody. Presence of some background noise:

Mouse sample (intestine)

Reference monoclonal anti-Ki67
1/250 ST



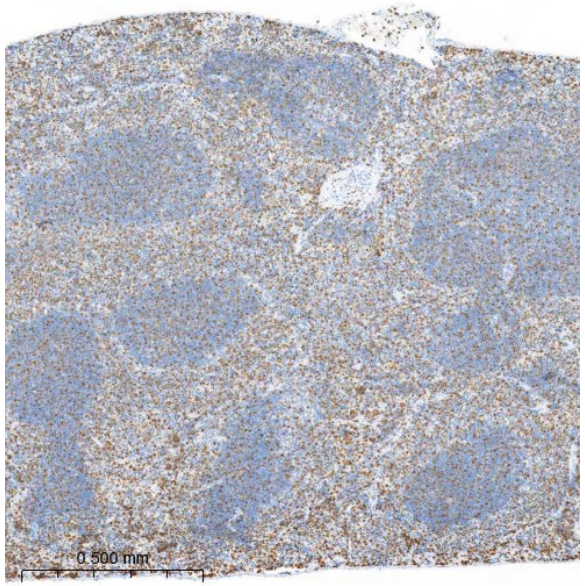
Recombinant monoclonal
anti-Ki67 Abcam Ab279653
1/100 ST

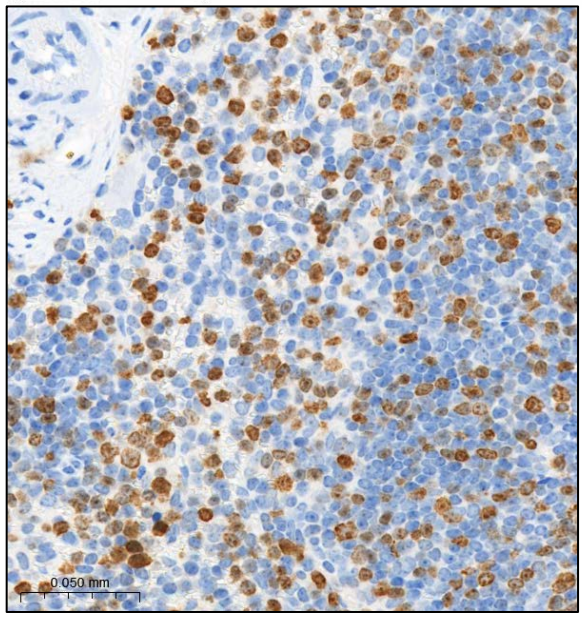


Signal more intense and less extensive than with the reference antibody. No background noise.

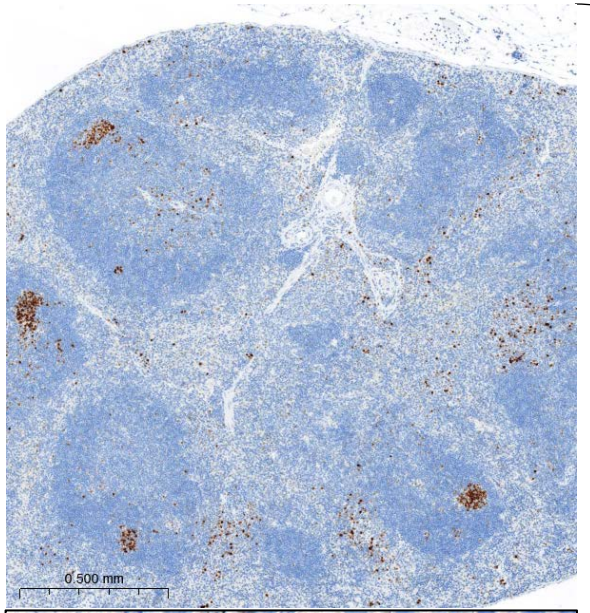
Mouse sample (spleen)

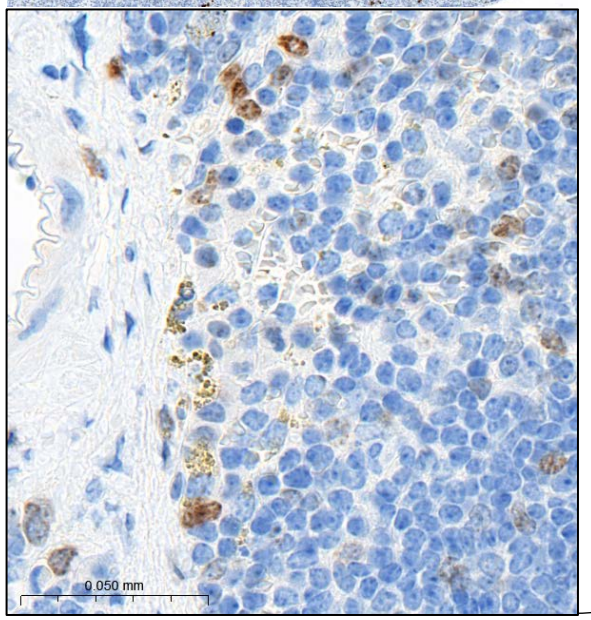
Reference monoclonal anti-Ki67
1/250 ST





Recombinant monoclonal
anti-Ki67 Invitrogen MA5-14520
1/250 ST

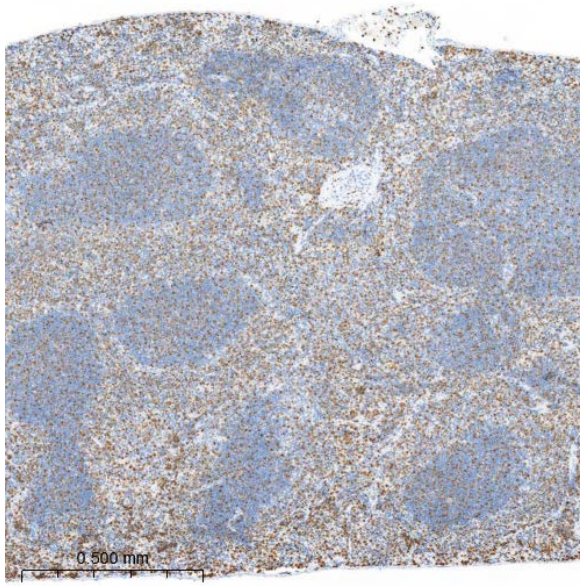


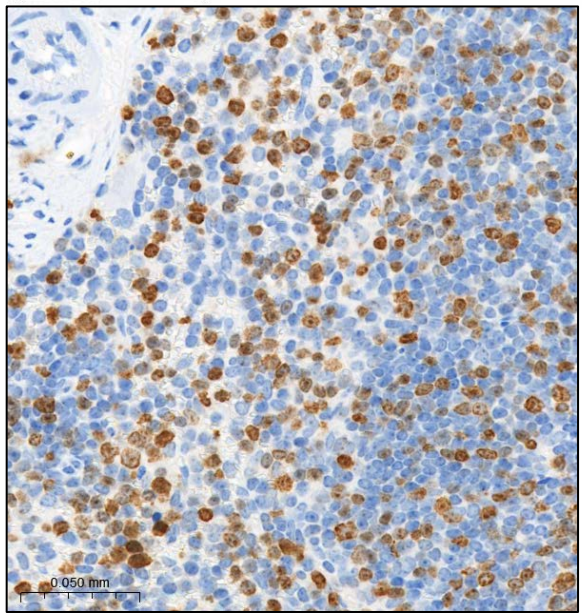


Clear signal, fewer cells stained than with the reference antibody.

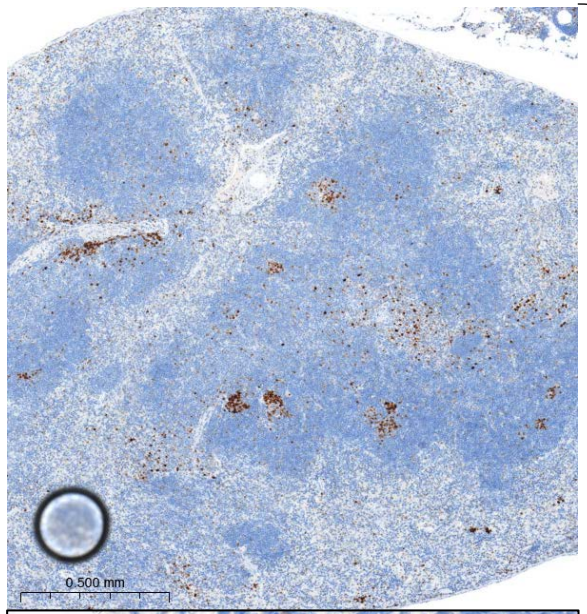
Mouse sample (spleen)

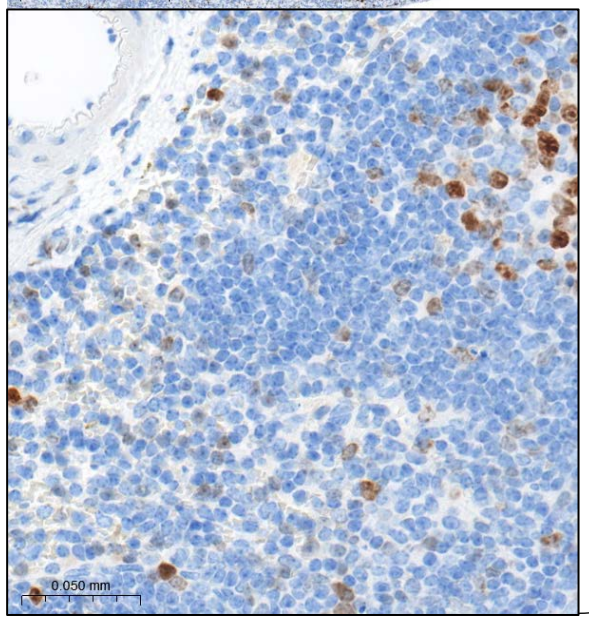
Reference monoclonal anti-Ki67
1/250 ST





Recombinant monoclonal
anti-Ki67 Abcam Ab16667
1/100 ST

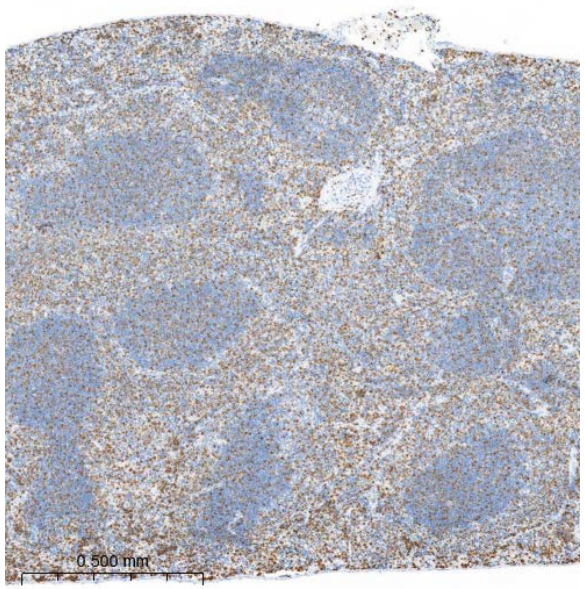


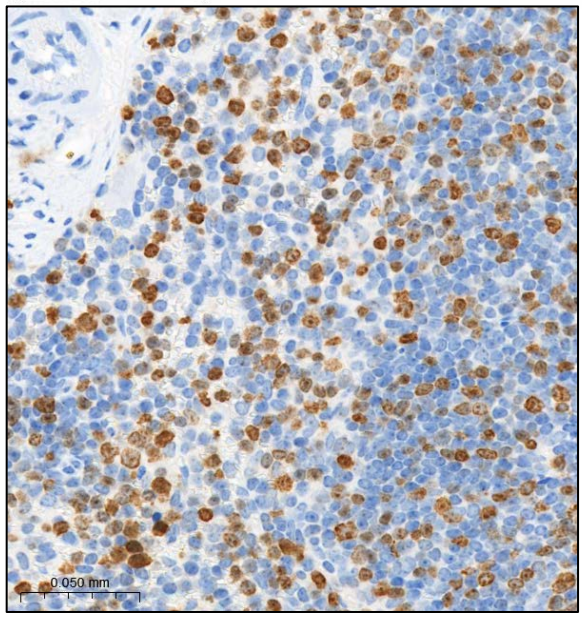


Clear signal, fewer cells stained than with the reference antibody.

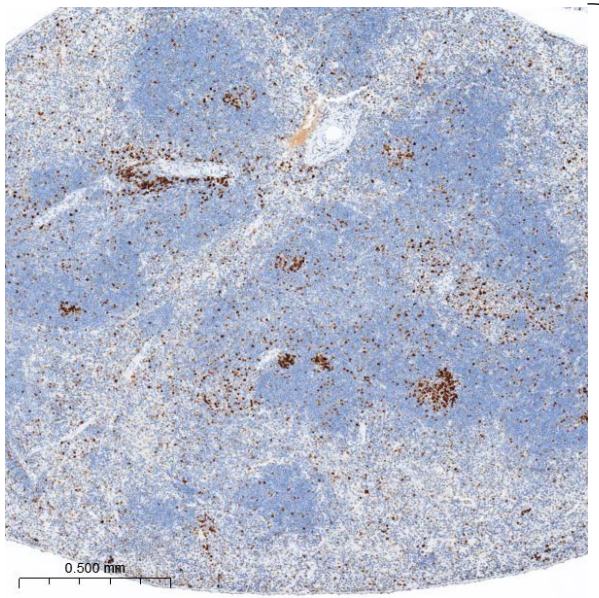
Mouse sample (spleen)

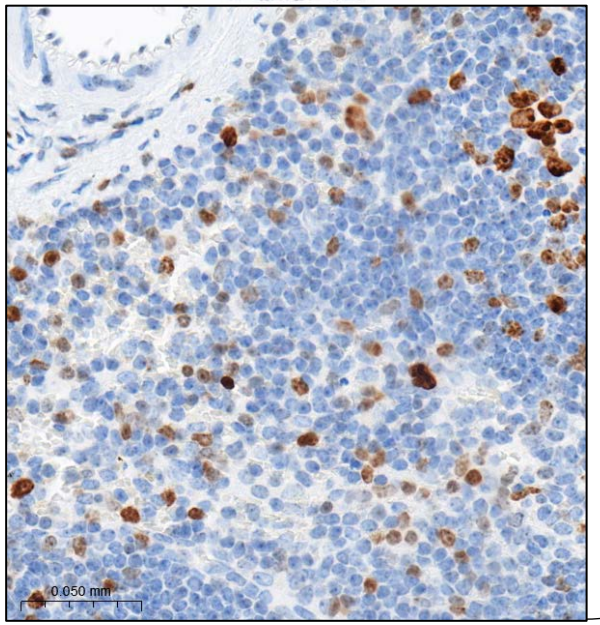
Reference monoclonal anti-Ki67
1/250 ST





Recombinant monoclonal
anti-Ki67 Abcam Ab279653
1/100 ST





Clear signal, fewer cells stained than with the reference antibody.

Materials and Methods

Experimental Strategy

The histology platform RHEM evaluated three recombinant anti-Ki67 antibodies on formalin-fixed paraffin-embedded (FFPE) tissue sections from mouse (intestine, spleen).

Initially, various conditions were tested for each antibody to determine optimal staining parameters. Once optimal conditions were established, antibody performance was assessed based on staining intensity, specificity, and background signal compared to a reference polyclonal anti-Ki67 antibody used by the platform. *NB : the reference polyclonal antibody used by the platform has been commercially discontinued.* Validation criteria included clear staining with minimal background.

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 (liver and skin) were obtained from *C57BL/6J* wild-type mice.

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, then dried at 37°C ON.

Antibody Testing

The tested rabbit recombinant monoclonal antibodies were :

- Anti-Ki67 - [SP6] - Invitrogen - *#MA5-14520* - Stock concentration: 0,031 mg/mL
- Anti-Ki67 - [SP6] - Abcam – *#ab16667* - Stock concentration: Not specified
- Anti-Ki67 - [B56] - Abcam – *#ab279653* - Stock concentration: 0,978 mg/mL

Immunohistochemistry was performed, as described previously (Rahmanzadeh, G. et al 2007), on a VENTANA Discovery Ultra automated staining instrument (Ventana Medical Systems), using VENTANA reagents, according to the manufacturer's instructions.

The recombinant anti-Ki67 antibodies were tested under these conditions :

Slides were de-paraffinized. Then epitope retrieval was performed with :

1. **CC1 (pH 8)** (*Roche, #05424569001*) : Heat-induced epitope retrieval (HIER) at 95°C for 24 min,
2. **CC2 (pH 6)** (*Roche, #05424542001*) : HIER at 91°C for 24 min.

If none of these 2 conditions worked, two other conditions were tested :

3. No antigen retrieval,
4. Protease 1 treatment (*Roche, #05266688001*) at 37°C for 4 min.

Endogenous peroxidase was blocked with Discovery Inhibitor (*Roche, #760-4840*) for 8 min.

The antibodies were diluted at 1/100^{e} in Antibody Diluent (*Agilent, ST, #S0809*) or Antibody Diluent with Background Reducing (*Agilent, LB, #S3022*).

All antibodies were incubated for 60 min at 37°C.

Signal was enhanced using the OmniMap anti-rabbit detection kit (*Roche, #05266548001*) for 16 min, according to the manufacturer's instructions.

Slides were incubated with DAB (*Roche, #05266645001*) then counterstained with hematoxylin II (*Roche, #790-2208*) for 8 min, followed by Bluing reagent (*Roche, #760-037*) for 4 min. Slides were then dehydrated with Leica autostainer and coverslipped with Pertex mounting medium with CTM6 coverslipper (Microm).

A reference monoclonal anti-Ki67 antibody (AMSBIO #M3064*, discontinued*) was used as a positive control:

- Rabbit monoclonal
- Concentration: stock 2 mg/mL ; working concentrations of 8 µg/mL
- Validated under the following conditions: **CC1 - 24 min - 95°C**, incubation at 37°C for 16 min, Hapten Multimer anti-Rabbit HQ and anti-HQ HRP detection (*Roche, #7017812001 & 7017936001)*, DAB chromogen

Image Acquisition

Images were acquired using a scanner Pannoramic MIDI II (3D Histech, MM France).

Results Summary

Three recombinant antibodies were validated to detect Ki67 in mouse.

However, the results are not comparable to the reference AMSBIO #M3064.

Supplier / Reference	Clone	Epitope retrieval	Dilution	Amplification
Abcam *ab16667*	SP6	CC1 - 24 min - 95°C	1/100[e] Diluent ST	OmniMap Rabbit
Abcam *ab279653*	B56	CC1 - 24 min - 95°C	1/200[e] Diluent ST	OmniMap Rabbit
Invitrogen *MA5-14520*	SP6	CC1 - 24 min - 95°C	1/250[e] Diluent ST	HQ Rabbit