# Echantillon souris (intestin)

Anti-Ki67 monoclonal de référence 1/250 ST



Anti-Ki67 Invitrogen MA5-14520 recombinant monoclonal 1/250 ST



Signal plus intense et moins étendu qu'avec l'anticorps de référence. Présence d'un peu de bruit de fond :

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Anti-Ki67 monoclonal de référence 1/250 ST



Anti-Ki67 Abcam Ab16667 recombinant monoclonal 1/100 ST



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# <mark>Echantillon souris (intestin)</mark>

Anti-Ki67 monoclonal de référence 1/250 ST



Anti-Ki67 Abcam Ab279653 recombinant monoclonal 1/100 ST





Signal plus intense et moins étendu qu'avec l'anticorps de référence. Pas de bruit de fond. Echantillon souris (rate)

Anti-Ki67 monoclonal de référence 1/250 ST



Anti-Ki67 Invitrogen MA5-14520 recombinant monoclonal 1/250 ST



Signal net, moins de cellules marquées qu'avec l'anticorps de référence.

Echantillon souris (rate)

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Signal net, moins de cellules marquées qu'avec l'anticorps de référence.

# Echantillon souris (rate)

# Anti-Ki67 monoclonal de référence 1/250 ST



Anti-Ki67 Abcam Ab279653 recombinant monoclonal 1/100 ST





Signal net, moins de cellules marquées qu'avec l'anticorps de référence.

## **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.

The sample 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 antibodies were :

- Ki67 [SP6] Rabbit recombinant monoclonal Invitrogen #MA5-14520
- Ki67 [SP6] Rabbit recombinant monoclonal Abcam ab16667
- Ki67 [B56] Mouse recombinant monoclonal Abcam ab279653

IHC 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. Endogenous peroxidase was blocked with Discovery Inhibitor (*Roche,* #760-4840). 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, antibody dilution

If these 2 conditions did not work :

- 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<sup>e</sup> 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 enhancement was performed 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) was used as a positive control:

- Rabbit monoclonal
- Concentration: stock 2 mg/mL; working concentrations of 8 ug/mL
- Validated under the following conditions: CC1 24 min 95°C, incubation at 37°C for 16 min, HQ detection, DAB chromogen

### **Image Acquisition**

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

#### **Results Summary**

All 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 <sup>e</sup>	OmniMap Rabbit
			Diluent ST	
Abcam ab279653	B56	CC1 - 24 min - 95°C	1/100 <sup>e</sup>	OmniMap Rabbit
			Diluent ST	
Invitrogen MA5-14520	SP6	CC1 - 24 min - 95°C	1/250 <sup>e</sup>	HQ Rabbit
			Diluent ST	