

Materials and Methods

Experimental Strategy

The histology platform RHEM evaluated four recombinant Fc Rabbit anti-CD31 antibodies on formalin-fixed paraffin-embedded (FFPE) tissue sections from mouse (spleen, lung, liver), rat (spleen, lung, liver), and human (liver, adipose tissue, colon).

Initially, various conditions were tested on mouse tissues (liver, lung, spleen) for each antibody to determine optimal staining parameters. Once optimal conditions were established in mouse tissues, they were subsequently tested on rat and human tissues. The JC70 antibody was also tested on human tissue despite failing in mouse, as it was developed against a human antigen.

Antibody performance was assessed based on staining intensity, specificity, and background signal compared to a reference polyclonal anti-CD31 antibody used by the platform. Validation criteria included clear endothelial membrane 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 were obtained from mouse (spleen, lung, liver), rat (spleen, lung, liver), and human (liver, adipose tissue, colon). The sample collected and fixed 24h to 48h in neutral buffered formalin 10%, dehydrated, and embedded in paraffin. Paraffin-embedded tissue was cut into 3- μ m-thick sections, mounted on slides, then dried at 37°C ON.

Antibody Testing

The tested antibodies were :

- Anti-CD31 [JC70] (Absolute Antibody) - Stock concentration: 1 mg/mL
- Anti-CD31 [AD_217] (ABCD Antibodies) - Stock concentration: 79 μ g/mL
- Anti-CD31 [Ab182981] (Abcam) - Stock concentration: 0.549 mg/mL
- Anti-CD31 [Ab281583] (Abcam) - Stock concentration: 0.505 mg/mL

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-CD31 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^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 enhancement was performed using the OmniMap anti-rabbit detection kit (*Roche*, #05266548001) for 16 min, or for antibodies with a weak signal, Discovery HQ conjugated antibody anti-rabbit IgG (*Roche*, # 07017812001) and Discovery amplification anti-HQ HRP Multimer (*Roche*, #06442544001) 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 (Micom).

A reference polyclonal anti-CD31 antibody (*Abcam* #Ab28364) was used as a positive control:

- Rabbit polyclonal
- Concentration: stock 0.013 mg/mL ; working concentrations of 0.17 µg/mL or 0.26 µg/mL (depending on the lot received)
- Validated under the following conditions: **CC1 (pH 8) 64 min at 95°C**, incubation at 37°C for 60 min, HQ detection, DAB chromogen

Image Acquisition

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

Results Summary

Two recombinant antibodies (Ab182981 and Ab281583) were validated in all three species. The JC70 antibody was validated in human tissue only. The AD_217 antibody did not produce specific staining under tested conditions.

Supplier / Reference	Clône	Epitope retrieval	Dilution	Amplification
Absolute Antibody #ab01338-23.0	JC70	CC2 – 24 min – 91°C	1/300 (3,33 µg/mL) Diluent LB	Rabbit HQ
Abcam #ab182981	EPR17259	CC1 - 24 min - 95°C	1/500 (1,098 µg/mL) Diluent ST	OmniMap Rabbit
Abcam #ab281583	RM1006	CC1 - 24 min - 95°C	1/500 (1,01 µg/mL) Diluent ST	OmniMap Rabbit

Recombinant antibodies significantly reduced the per-slide cost (1.34€/slide) compared to the polyclonal reference antibody (8.57€/slide), representing up to a six-fold cost reduction.