Materials and Methods

Experimental Strategy

The histology platform APEX evaluated three recombinant Fc Rabbit anti-GFAP antibodies on formalin-fixed paraffin-embedded (FFPE) brain sections from C57Bl6 mouse (APEX ID 2400674), Fischer rat (APEX ID 2400601) and *Macaca fascicularis* (APEX ID 180205).

Initially, various conditions were tested on mouse tissue for each antibody to determine optimal staining parameters. Once optimal conditions were established in mouse tissues, they were subsequently tested on rat and PNH tissues.

Antibody performance was assessed based on staining intensity, specificity, and background signal compared to a reference polyclonal anti-GFAP antibody used by the platform. Validation criteria included strong cytoplasmic staining revealing an intricate network of fine, filamentous structures outlining the star-shaped morphology of astrocytes, with processes radiating from the cell bodies.

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, 3 serial sections 4 µm-thick were immunolabelled with primary antibodies against GFAP

Antibody Testing

The tested antibodies were:

- Abcam ref ab278054 = Rabbit recombinant multiclonal (RM1003) (470€); Stock concentration: [471 µg/mL]
- ABCD antibodies ref AK148 = Rabbit recombinant monoclonal (217€); Stock concentration: [94 µg/mL]
- Ozyme ref 80788T = GFAP (E4L7M) XP® Rabbit recombinant monoclonal (226€) ; Stock concentration: [62 µg/mL]

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

Pretreatment :

1. No antigen retrieval
2. **CC1** Tris-EDTA pH 8 (*Roche diagnostics, 950-500*) at 95°C for 40 min
3. **CC2** Citrate pH 6 (*Roche diagnostics, 950-223*) at 91°C for 40 min

Tested dilutions :

- Abcam ab278054 : 1:1000 and 1:2000
- ABCD AK148 : 1:100 and 1:200

- Ozyme 80788T : 1:100 and 1:200

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 760-159)* 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 760-159)* 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 Z0334 = polyclonal Rabbit IgG (1:2000) ; with CC1 pretreatment.

Image Acquisition

Images were acquired using a scanner Zeiss Axioscan Z1.

Results Summary

All tested references displayed best or similar immunolabelling properties compared to the reference antibody.

Validated conditions :

	Dilution	Pretreatment
Abcam ab278054	1:2000	CC1
ABCD AK148	1:100	CC1
Ozyme 80788T	1:200	CC1