Immunohistochemistry Protocols

IHC was performed on 2 µm thick sections of FFPE tissue blocks using the primary anti-FAP antibody ab207178 (EPR20021; Abcam) diluted 1:100 for human, 1:200 for canine and 1:150 for feline tissue.

Tissue sections were mounted on positively-charged slides (SuperFrost Plus slides, Thermo Fisher Scientific) and dried overnight at 37°C. Unstained sections were deparaffinized with four xylene baths for 5 min each using the Tissue-Tek Film (Sysmex) followed by rehydration using degressive alcohol series (100%, 95%, 70% 70% ethanol) and rinsing in distilled water. Slides were incubated in EDTA-buffer (pH 9.0) for 20 minutes in a pressure cooker set to 98 °C for heat induced antigen retrieval, followed by a washing step in distillated water. Slides were put into wash-buffer TBS (Dako 3006) before being stained in the Dako -Autostainer. The primary antibody was diluted, and incubation was performed for 60 min at room temperature (RT) for canine tissue and over night for feline tissue, followed by peroxidase blocking (peroxidase blocking buffer, Dako S2023) for 10 min at RT, and incubation with the secondary antibody (Envision+System HRP Rabbit (Dako K4003)) for 30 min at RT. Between those steps slides were rinsed with wash-buffer TBS (Dako 3006). For visualization, the DAB Detection Kit (Dako K3468) was used for an incubation time of 10 min at RT followed by rinsing with distilled water. All sections were counterstained with hematoxylin for 2 seconds, rinsed with tap water, dehydrated in the Prisam (Sysmex) with increasing Xylol series (70%, 95%, 100%, Xylol) and coverslipped with the Tissue-Trek-Film.

<u>Control tissue</u>

In dogs and cats used control tissues samples comprised healthy skin, a canine STS and a feline mammary carcinoma, and for humans a mammary carcinoma. As negative control tissue in dogs and cats a healthy skin sample and in humans was used as well as omission of the primary antibody on the positive control.