Materials and Methods

Experimental Strategy

The histology platform RHEM evaluated four recombinant Fc Rabbit anti-GFP antibodies on formalin-fixed paraffin-embedded (FFPE) tissue sections from mouse (liver, skin) expressing Green Fluorescent Protein (GFP) or Yellow Fluorescent Protein (YFP).

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-GFP antibody used by the platform. Negative controls (GFP- or YFP-) were included. 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 mouse of the following genetic backgrounds :

	Positive sample	Negative control	
GFP	Mus musculus domesticus, 2.5 months,	Mus musculus domesticus, 3.75	
	Male	months, Male	
	Genotype:	Genotype:	
	B6 / p53 KOc f/f ; Mdm2 KOc f/f ; E4F1	B6 / p53 KOc f/f ; Mdm2 KOc +/+ ;	
	KOc +/+ ; Rosa-CRE-ERTam KI +/CRE-	E4F1 KOc f/f; Rosa-CRE-ERTam	
	ERTam ; mTmG-mTmG KI +/mTmG	KI +/+ ; mTmG-mTmG KI +/mTmG	
	Treatment: Tamoxifen for 5 days (2 mg	Treatment: Tamoxifen administered	
	per mouse)	for 5 days (2 mg per mouse)	
YFP	Mus musculus domesticus, 2 months,	Mus musculus domesticus, 2	
	Male	months, Male	
	Genotype:	Genotype:	
	B6 / p53-R172H KI +/R172H ; YFP-YFP	B6 / p53-R172H KI +/R172H ; YFP-	
	KI +/YFP ; ROSA-CRE-ERTam KI	YFP KI +/YFP ; ROSA-CRE-	
	+/CRE-ERTam ; p53 KOc +/f	ERTam KI +/CRE-ERTam ; p53	
		KOc +/f	

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-GFP Fc Rabbit [AK652] (ABCD Antibodies) - Stock concentration: 120 µg/mL

- Anti-GFP Fc Rabbit [#2956] (Cell Signaling) Stock concentration: 7 µg/mL
- Anti-GFP Fc Rabbit [Ab183734] (Abcam) Stock concentration: 0,138 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-GFP 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, 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 polyclonal anti-GFP antibody (*Invitrogen #A-11122*) was used as a positive control:

- Rabbit polyclonal
- Concentration: stock 2 mg/mL; working concentrations of 8 ug/mL
- 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 Pannoramic MIDI II (3D Histech, MM France).

Results Summary

All three recombinant antibodies were validated to detect GFP in the transgenic mice tissues. Only the #2956 from Cell Signaling recombinant antibody was validated to detect YFP in transgenic mice tissues.

Supplier / Reference	Clone	Epitope retrieval	Dilution	Amplification
ABCD Antibodies	/	Protease 1 - 4 min - 37°C	300 ng/mL	OmniMap Rabbit
AK652			Diluent LB	
Cell Signaling	D5.1	CC1 (pH8) - 24min - 95°C	35 ng/mL	OmniMap Rabbit
#2956			Diluent ST	
Aboom Ab182724	EPR14104	CC1 (pH8) - 24min - 95°C	34,5 ng/mL	OmniMap Rabbit
Autain Autos/34			Diluent ST	