Materials and Methods

Experimental Strategy

The histology platform from Inrae St Gilles evaluated four recombinant Fc Rabbit anti-KRT14 antibodies on formalin-fixed cells and paraffin-embedded (FFPE) mammary gland sections from cow, goat and sow.

Initially, various conditions were tested on bovine cell culture expressing KRT14 (biological positive control) for each antibody to determine optimal staining parameters. Once optimal conditions were established, they were subsequently tested on cow, goat and sow tissues.

Antibody performance was assessed based on staining intensity, specificity, and background signal. When possible, they were compared to the reference polyclonal anti-KRT14 antibody used by the platform ; however, this reference antibody does not detect goat and porcin KRT14. Validation criteria in tissues included a clear staining of the cells bordering the mammary alveoli.

Biological Material

All formalin-fixed paraffin-embedded (FFPE) samples were already available on the platform. No animal has been sacrificed for these tests.

- Paraffin-embedded sections of cow mammary gland (17-month-old heifer)
- Bovine mammary epithelial cells (MAC-T cell line) cultured on inserts
- Paraffin-embedded sections of goat mammary gland (prepubertal)
- Paraffin-embedded sections of pig mammary gland (lactating sow)

Antibody Testing

The antibody of animal origin (Santa Cruz; cross-reactive antibody for bovine species) was tested against four recombinant antibodies:

Supplier	Reference	Clone	Fc domain	Concentration	Dilution
Santa Cruz	Sc-17104	C-14	Goat IgG	0.2 mg/ml	1:100
Abcam	ab119695	SP53	Rabbit IgG	1.17 mg/ml	1:250
Cell Signaling	CST 74956	E7W6V	Rabbit mAb	50 µg/ml	1:200
Absolute Antibodies	Ab03180-23.0	2G4	Rabbit IgG, kappa	1 mg/ml	1:250
Absolute Antibodies	Ab03383-23.0	RCK107	Rabbit IgG, kappa	1 mg/ml	1:200

The recombinant anti-KRT14 antibodies were tested under these conditions:

Epitope retrieval
EDTA buffer vs. TRIS 1 mM pH 8 buffer

Protocol for Cell Staining (on Inserts)

- Cells on inserts were fixed with 4% PFA (*VWR, ref. FOR0060AF59001*) for 30 min at room temperature (1 ml of PFA per compartment), then washed twice for 5 min with PBS. For each step, 1 ml of solution (other than antibody and Hoechst solutions) was added to both compartments (basolateral and apical) of the insert. Washing consisted of incubating the cells for 5 min in PBS.
- Cells were permeabilized for 5 min with PBS containing 0.5% Triton X-100 (*Merck #1.08603.1000*), then washed twice. After a 30-min blocking in PBS with 2% BSA (*Merck #1.12018.0100*) followed by a wash, cells were incubated for 1 h 30 min at 37°C with primary antibody solution (100 µl per insert) diluted in PBS with 0.2% BSA, freshly prepared. Before antibody incubation, the insert was placed on the plate lid to ensure a hard surface.
- The insert was then returned to its well and washed twice. Cells were incubated for 45 min at 37°C with secondary antibody solution diluted in PBS with 0.2% BSA, following the same protocol as for primary antibodies. Cells were washed twice, and nuclei were stained with Hoechst (*Merck #14533*) at 5 µg/ml (100 µl per insert) for 3 min, followed by two PBS washes and a final wash in Milli Q water.
- For each insert, the membrane was cut and mounted on a slide with mounting medium and coverslip

Protocol for Paraffin-Embedded Tissue Sections

- Sections were deparaffinized using a standard program.
- Slides were washed twice for 5 min in TBS (20 mM Tris-base, 150 mM NaCl, pH 7.6). Washing consisted of incubating slides for 5 min in TBS.
- An epitope retrieval step was performed with TRIS 1 mM (pH 8) buffer or EDTA buffer (immunoDNA retriever EDTA 20X, BSB0033, Bio SB) using the TintoRetriever (BioSB) device with the parameters of pressure at 110°C for 15 min.
- After two washes, a blocking step was carried out. Slides were incubated1 h with blocking buffer (TBS, 10% horse serum, 3% Triton X-100).
- Subsequently, tissue sections were incubated with primary antibodies (100 µl/section; see antibody list) diluted in blocking buffer for 1 h 30 min at 37°C then washed twice.
- Following washing, tissue sections were incubated with the relevant secondary antibodies for 45 min at 37°C (100 µl/section): anti-goat Alexa Fluor 488 (Life Technologies #A11055) or anti-rabbit Alexa Fluor 488 (Life Technologies #A11008) diluted in blocking buffer.
- After two washing, nuclei were stained with 5 µg/ml bisbenzimide H33342 or Hoechst (14533, Merck) for 3 min.
- Slides were washed twice followed by a final 5-min rinse in Milli Q water.
- Tissue sections were dried and mounted with fluoromount and coverslip

Image Acquisition

Images were acquired using an Apotome™ microscope and the Zen acquisition software (Zeiss France).