Materials and Methods

Experimental Strategy

The histology platform from Inrae St Gilles evaluated two recombinant anti-CHGA antibodies on formalin-fixed cells and paraffin-embedded (FFPE) intestinal sections from mouse, rat and pig.

Antibody performance was assessed based on staining intensity, specificity, and background signal. They were compared to the reference polyclonal anti-CHGA antibody used by the platform. Validation criteria in tissues included robust detection of Chromogranin A in enteroendocrine cells across multiple intestinal tissue sections.

Biological Material

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

- **Tissue Samples:**
  Paraffin-embedded tissue sections (5 µm thickness) were prepared from the ileum, colon, jejunum and caecum of three species: pig, rat and mouse.

- **Sectioning:**
  Sections were cut at 5 µm thickness and mounted on glass slides for immunofluorescence staining.

Antibody Testing

- **Primary Antibodies:**

- Rabbit Anti-Chromogranin A polyclonal antibody (Abcam, ab45179), used at 1:400 (routine positive control)
- Rabbit Anti-Chromogranin A recombinant multiclonal antibody (Abcam, ab283265), used at 1:400
- Mouse Anti-Chromogranin A recombinant monoclonal antibody (Cell Signaling, 36468), used at 1:400

- **Secondary Antibodies:**

- Goat Anti-rabbit IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 Conjugate, Ozyme, 4412S), used at 1:500
- Goat Anti-mouse IgG (H+L), $F(ab')_2$ Fragment (Alexa Fluor® 488 Conjugate, Ozyme, 4408S), used at 1:500

- **Staining Protocol:**

1. **Deparaffinization:**

Automated deparaffinization program, 30 min

2. **Antigen Retrieval:**

20X ImmunoDNA Retriever Citrate solution (diluted 1:20 in PBS 1X) for 15 min at 110°C in a TintoRetriever Pressure Cooker (low pressure)

3. **Washing:**

Three washes in PBS 1X, 5 min each

4. **Permeabilization/Blocking:**

30 min at room temperature in blocking buffer (10% horse serum and 0.3% Triton X-100 in PBS 1X)

5. **Primary Antibody Incubation:**

30 min at room temperature

6. **Washing:**

Three washes in PBS 1X, 5 min each

7. **Secondary Antibody Incubation:**

1 h at room temperature

8. **Washing:**

Three washes in PBS 1X, 5 min each

9. **Mounting:**

Slides were mounted with Mounting Medium With DAPI - Aqueous, Fluoroshield (Abcam, ab104139)

Image Acquisition

- **Microscope:**

Axio Imager M2 with Apotome 2 module (Zeiss)

- **Objective:**

20x magnification

- **Imaging:**

Slides were imaged under fluorescence illumination, and images were acquired using appropriate filters for Alexa Fluor 488 and DAPI

Results

- No signal was observed with the mouse anti-Chromogranin A antibody from Cell Signaling (36468) under these conditions

- Strong and specific immunofluorescence was observed with the rabbit anti-Chromogranin A antibody from Abcam (ab283265)