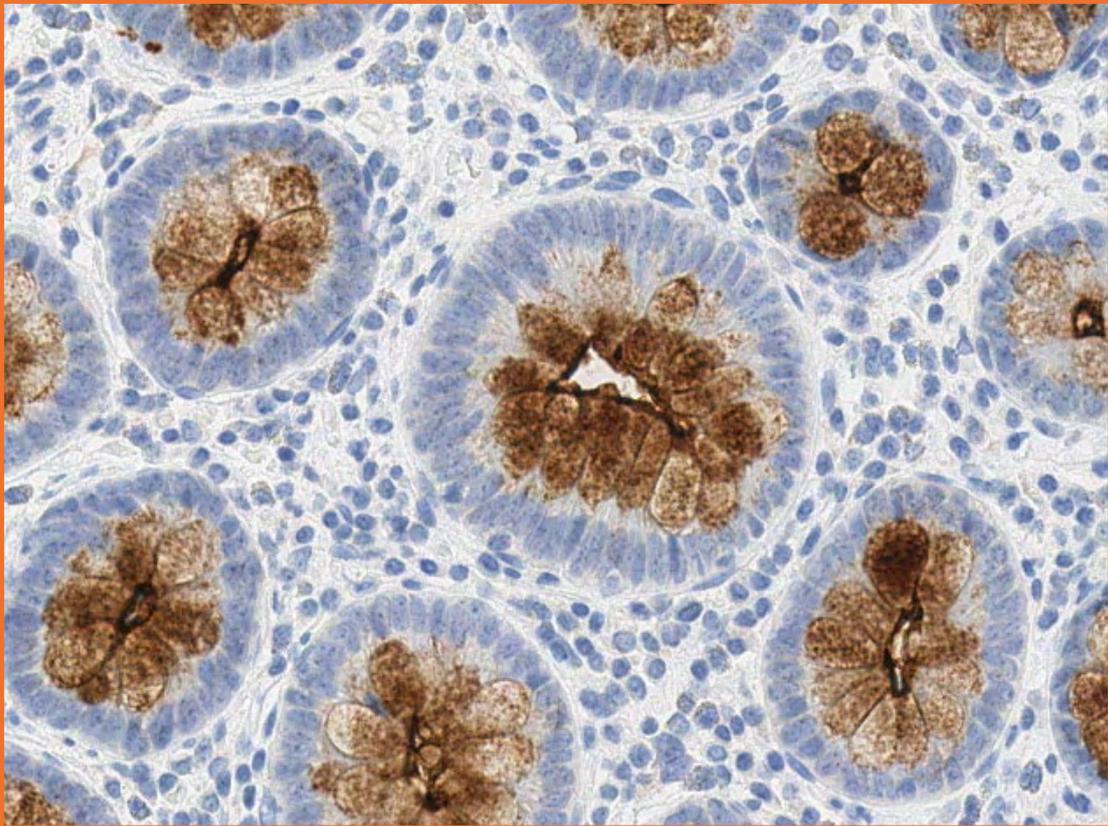


# Enhanced validation data

## Anti-MUC4 recombinant antibody – ab307546



# Enhanced validation of Anti-MUC4 recombinant antibody [EPR27199-56] – ab307546

## Enhanced validation designed for your needs

We understand the challenge of finding the right antibody clone – highly specific and sensitive to your intended target – at early selection stages of your development program. To de-risk this clone selection process for you, we generated enhanced validation data for our best recombinant antibody clones to some of the most promising targets.

**Our enhanced validation gives you an extra level of confidence** in an antibody clone

- Provides additional data on the specificity and sensitivity of our recombinant antibodies in immunohistochemistry (IHC) and other relevant techniques
- Carried out in a custom manner, specific both to the target and the relevant research & clinical settings
- Builds upon our high-quality standard validation

## Our framework for enhanced validation

- Our enhanced validation focuses on generating detailed IHC expression profiles for promising oncology targets in selected formalin-fixed paraffin-embedded (FFPE) human normal tissues and cancer tissue microarrays (TMAs).
- In this study, we demonstrate the sensitivity and specificity of Anti-MUC4 antibody (ab307546) in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®) and DISCOVERY ULTRA system (Roche Diagnostics).
- Alcian blue stain, a special stain technique for mucin evaluation, was used in parallel with the IHC assay to demonstrate antibody specificity.
- A multiplex (duplex) assay was also developed using the DISCOVERY ULTRA system (Roche Diagnostics).
- Staining intensity analysis of MUC4 expression was performed using the artificial intelligence (AI)-driven digital image analysis software Visiopharm® (Visiopharm A/S).

## Target overview

### HGNC symbol

MUC4

### Approved name

Mucin 4, cell surface associated

### Chromosomal location

3q29

## Function

- Provides protection to the epithelium and promoting epithelial regeneration and differentiation<sup>1</sup>.
- Through its both anti-adhesive effects on cell-cell and cell-extracellular matrix interactions, MUC4 regulates the cellular behaviour. Tumor cells dissociate from the primary tumor site due to MUC4's anti-adhesive activity, which triggers epithelial invasion<sup>2,3</sup>.
- Plays an important role in cellular survival mechanism<sup>4</sup> and therapeutic resistance<sup>5,6</sup>.

## Tissue specificity

- MUC4 is detected in various adult epithelial tissues, including those of the respiratory and digestive tracts, esophagus, stomach, small intestine and colon<sup>7-10</sup>.
- Expressed in carcinomas arising from some of these epithelia such as lung cancers, squamous cell carcinomas of the upper aerodigestive tract, mammary carcinomas, biliary tract, colon, and cervix cancers<sup>5, 11-13</sup>.

## Cellular localization

- Cell membrane; also secreted.

Target information above in part from: UniProt accession Q99102

The UniProt Consortium

The Universal Protein Resource (UniProt) in 2010

[Nucleic Acids Res. 38:D142-D148 \(2010\)](#)

## Materials and methods

Human tissues were selected based on the target's expression and its current relevance to ongoing research and clinical trials. Gene expression was further analyzed for oncology targets in cBioPortal for Cancer Genomics using the Cancer Genome Atlas (TCGA) PanCancer Atlas datasets<sup>14-17</sup>.

Tissue microarray (TMA)	Cores	Cases	Normal/ Benign cases	Cancer cases	Source (#catalog number)
Multi-normal <sup>(a)</sup>	40	37	37	0	In-house TMA
Multi-cancer <sup>(b)</sup>	40	35	1	34	In-house TMA
Cervix cancer	102	102	5	97	Pantomics (#CXC1021)
Colon cancer	102	102	22	80	Pantomics (#COC1021)
Pancreatic cancer	102	96	6	96	Pantomics (#PAC1021)
Breast cancer	96	48	0	48	Pantomics (#BRC962)

**Table 1. List of human TMAs used in the enhanced validation.** All tissues were sourced from Abcam-approved tissue suppliers.

a) The multi-normal TMA consists of the following tissues from two donors: colon, cerebellum, small intestine mucosa, tonsil, stomach, testis, prostate, lung, skeletal muscle, breast, heart, skin, endometrium, spleen, pancreas, lymph node, kidney. Placenta and liver was from a single donor.

b) The multi-cancer TMA consists of the following tissues from two donors: seminoma, prostate adenocarcinoma, bladder carcinoma, renal cell carcinoma, melanoma, stomach adenocarcinoma, pancreatic adenocarcinoma, hepatocellular carcinoma, ovaria carcinoma, cervical cancer, head and neck carcinoma and endometrial cancer. The following tissues were from single donors: lung (squamous cell carcinoma (SCLC) and non-squamous cell carcinoma (NSCLC)), colon (adenocarcinoma and invasive adenocarcinoma), breast (ductal carcinoma and invasive lobular carcinoma), B-cell lymphoma, T-cell lymphoma, gliomas (grade II and IV) and placenta.

Step	Reagents	Method
Deparaffinization	DISCOVERY Wash (RUO)	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	64 min, 100°C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Anti-MUC4 antibody [EPR27199-56] – ab307546 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 1.5 µg/mL	16 min, 37°C
Counterstain	Hematoxylin II	8 min
Post counterstain	Bluing Reagent	4 min

**Table 2 . IHC staining protocol on the DISCOVERY ULTRA (Roche Diagnostics) instrument.** Staining was performed using standard conditions with OptiView DAB IHC Detection kit (#760-700).

## Enhanced validation data

Step	Reagents	Method
Deparaffinization	DISCOVERY Wash (RUO)	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	64 min, 95°C
DISC inhibitor	DISCOVERY Inhibitor (#760-4840)	8 min
1 <sup>st</sup> Primary antibody	Anti-MUC4 antibody [EPR27199-56] – ab307546 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 1.5 µg/mL	16 min, 37°C
1 <sup>st</sup> Linking antibody	DISCOVERY Anti-Rb HQ (#760-4815)	12 min, 37°C
1 <sup>st</sup> Enzyme conjugate	DISCOVERY Anti-HQ HRP (#760-4820)	12 min
1 <sup>st</sup> HRP-driven chromogen	DISCOVERY Purple kit (RUO) (#760-229)	12 min
Dual sequence antibody Denaturation	ULTRA Cell Conditioning Solution (ULTRA CC2)	8 min, 100°C
2 <sup>nd</sup> Primary antibody	Anti-ErbB2 / HER2 antibody [EP1045Y] - ab134182 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 1.0 µg/mL	16 min, 37°C
2 <sup>nd</sup> Linking antibody	DISCOVERY Anti-Rb HQ (#760-4815)	12 min, 37°C
2 <sup>nd</sup> Enzyme conjugate	DISCOVERY Anti-HQ HRP (#760-4820)	12 min
2 <sup>nd</sup> HRP driven chromogen	DISCOVERY Teal kit (RUO) (#760-247) Teal HRP H202	4 min
	DISCOVERY Teal kit (RUO) (#760-247) Teal HRP Act	12 min
Counterstain	N/A	-

**Table 3. Duplex IHC staining protocol on the DISCOVERY ULTRA (Roche Diagnostics) instrument.** Staining was performed using standard conditions with DISCOVERY Purple kit (RUO) (#760-229) and DISCOVERY Teal HRP kit (#760-247). These translucent chromogens shift in color when both are present in the same cell and sub-cellular compartment. Co-localized DISCOVERY Purple and DISCOVERY Teal combine to form an indigo-blue-to-deep-purple color.

## Enhanced validation data

Step	Reagents	Number of washes	Time (minutes)
Deparaffinization	Histoclear	3x	2
	95% Ethanol	3x	2
	Running dh <sub>2</sub> o	1x	5
Acetic acid treatment	Acetic acid	1x	3
Alcian Blue treatment	Alcian Blue pH2.5	1x	20
Wash	Running dh <sub>2</sub> o	4x	-
Counterstain	Nuclear Fast red	1x	5
Wash	Running dh <sub>2</sub> o	4x	-
Dehydrate and clear	95% Ethanol	3x	2
	Histoclear	3x	2

**Table 4. Alcian Blue staining protocol.** The alcian Blue, pH 2.5 (mucin stain) (ab150662) is intended for use in the histological visualization of sulfated and carboxylated mucopolysaccharides and sulfated and carboxylated sialomucins (glycoproteins).

## Enhanced validation data

Step	Reagents	Method
Dewax	Bond™ dewax solution (AR922), alcohol, BOND wash solution (AR9590)	Dewax
Antigen retrieval	Bond™ epitope retrieval ER2 solution (AR9640)	HIER with ER2 (pH 9.0), 20 min, 100°C

Step	Reagents	Number of washes	Time (minutes)
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Anti-MUC4 antibody [EPR27199-56] – ab307546 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 1.5 µg/mL	-	15
Wash	Bond™ wash solution	4x	0
Secondary antibody	Bond™ polymer refine detection (DS9800)	-	8
Wash	Bond™ wash solution	2x	4
	Deionized water	1x	0
Visualization	Mixed DAB refine (DS9800)	1x	0
	Mixed DAB refine (DS9800)	-	10
Wash	Deionized water	3x	0
Counterstain	Hematoxylin (DS9800)	-	5
	Deionized water	1x	0
Wash	Bond™ wash solution	1x	0
	Deionized water	1x	0

**Table 5. IHC staining protocol on BOND™ RX Research Stainer (Leica®).** The protocol used is the same as the default IHC protocol F on BOND™ RX Research Stainer (Leica®), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature.

Leica® is a registered trademark of Leica Microsystems IR GmbH.  
BOND™ is a trademark of Leica Biosystems Melbourne Pty. Ltd.

## Evaluation of staining intensity

MUC4 staining intensity analysis was performed using the artificial intelligence (AI)- driven digital image analysis software Visiopharm® (Version: 2023.09). TMA slides were de-arrayed and the tissue within each core was detected. Tissue detection and artefact exclusion were performed using models with DeepLabv3+ architecture. The graphical representation was generated using GraphPad Prism 10.

IHC staining	Corresponding intensity score	Relative average DAB intensity (AU)
Negative	0	0-10
Weak	1+	< 80
Moderate	2+	80-160
Strong	3+	> 160

**Table 6. Intensity scoring.** The mean DAB intensity was measured for each core with an analytical range from 0 (+) to 250±2 (-) where a low mean DAB intensity score inversely corresponds to high expression. For the graphical representation, the relative average DAB intensity was calculated using the formula (Relative average DAB intensity= 250±2 - mean DAB intensity) and is represented in arbitrary units (AU).

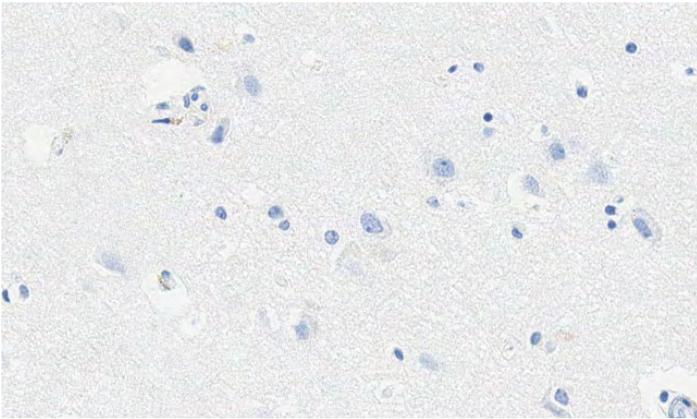
Visiopharm® is a registered trademark of Visiopharm A/S.

## MUC4 expression in multi-normal TMA (DISCOVERY ULTRA)

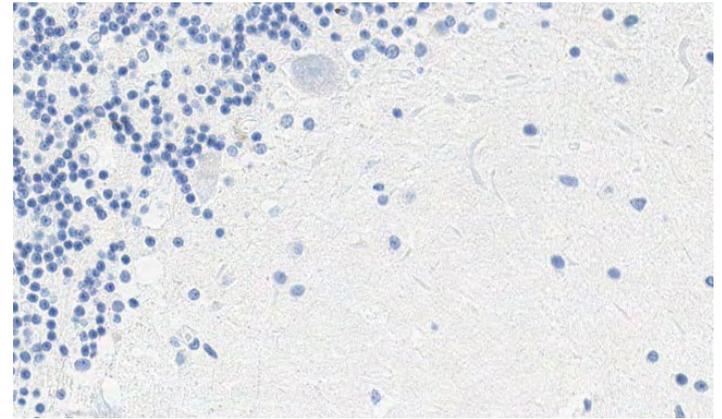
Below are the representative images of selected tissues from multi-normal TMA. MUC4 expression was detected in the small intestine and colon and was very minimal to absent in the brain, kidney, skeletal muscle, spleen, endometrium, pancreas, testis, and placenta.

### MUC4

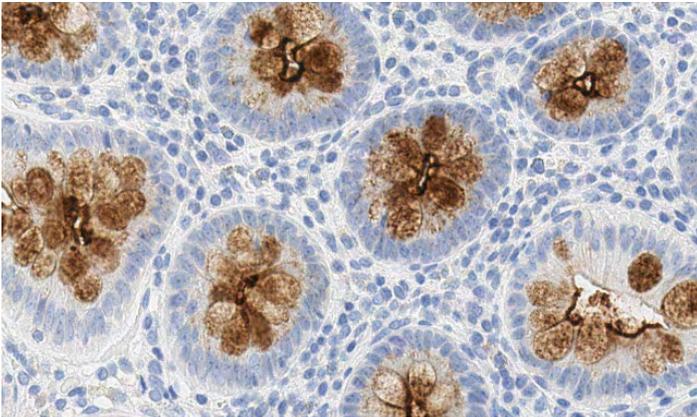
Brain-cerebrum



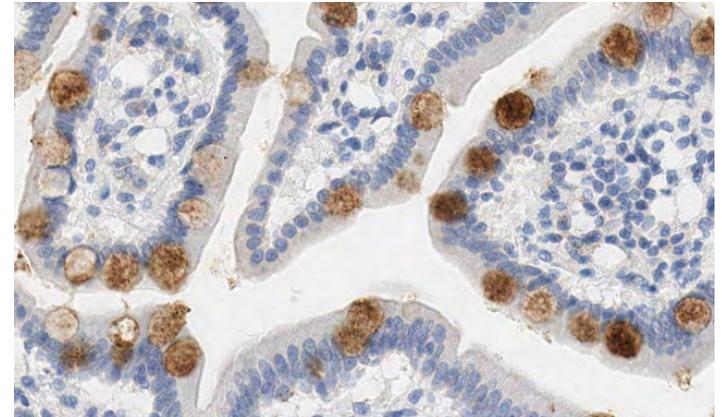
Brain-cerebellum



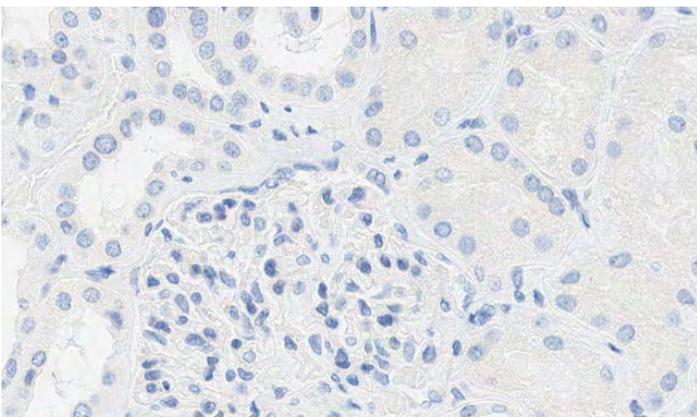
Small intestine



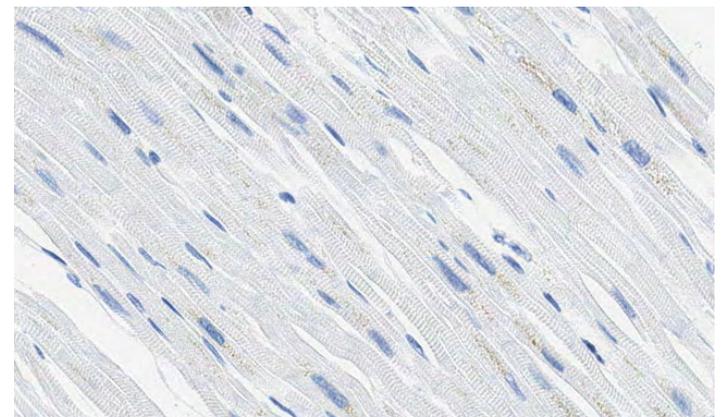
Colon



Kidney

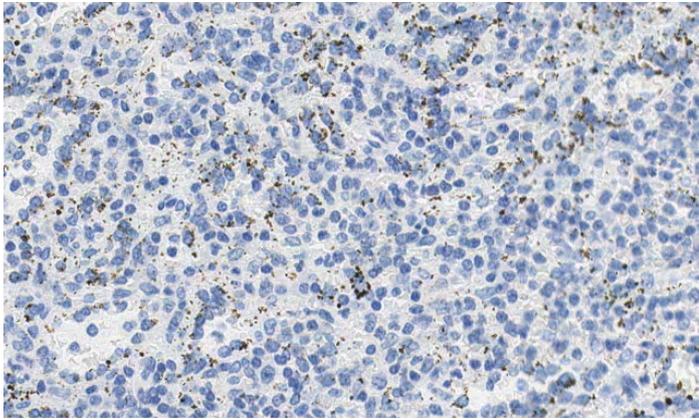


Skeletal muscle

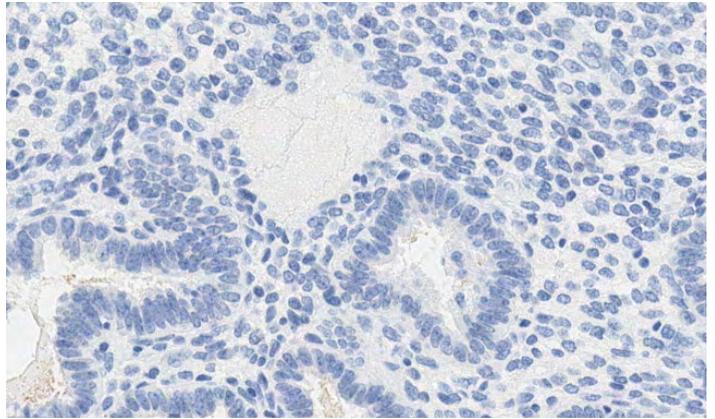


## MUC4

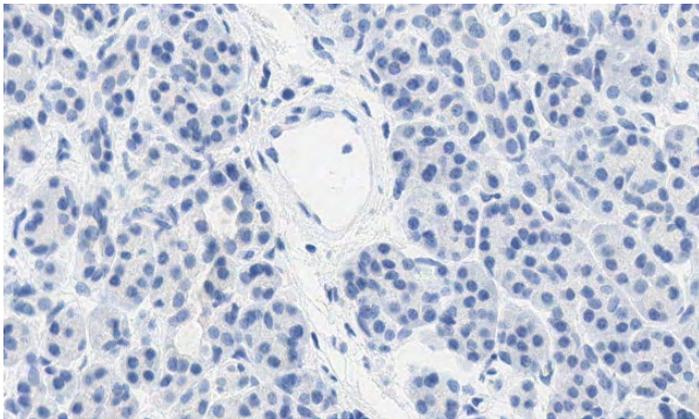
Spleen



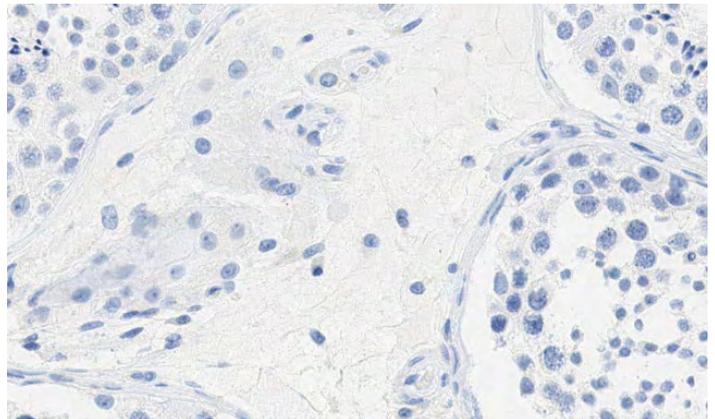
Endometrium



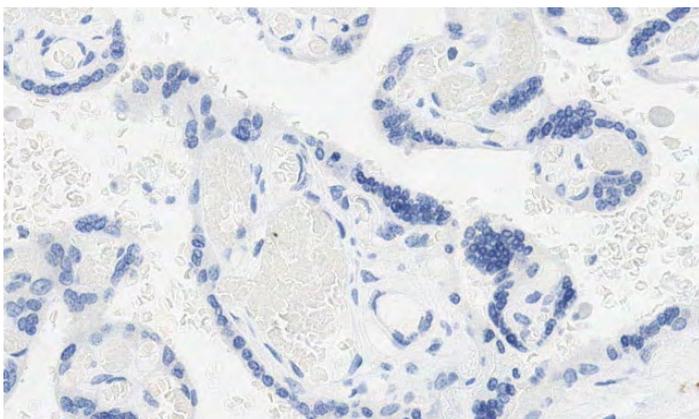
Pancreas



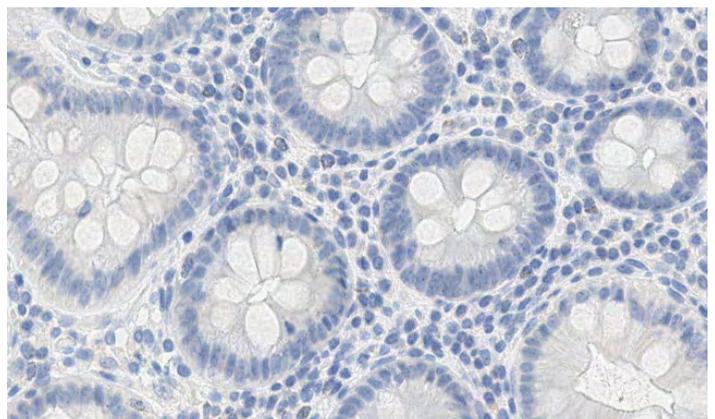
Testis



Placenta



Isotype control - Small intestine



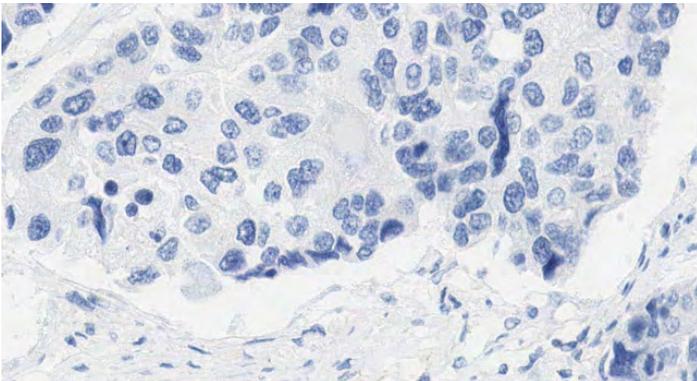
**Figure 1. MUC4 expression in human normal tissue.** IHC staining of multi-normal human tissues using anti-MUC4 (ab307546) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## MUC4 expression in multi-cancer TMA (DISCOVERY ULTRA)

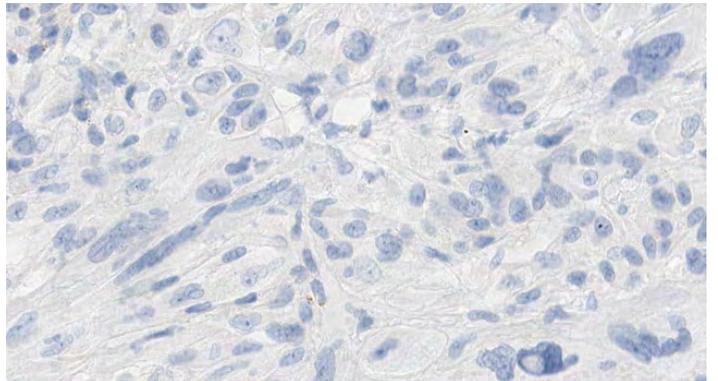
Below are the representative images of selected tissues from multi-cancer TMA. MUC4 expression was detected in stomach adenocarcinoma, non-small cell lung carcinoma, cervical cancer (adenocarcinoma and squamous cell carcinoma). Expression was absent in breast fibroadenoma, glioblastoma, seminoma, renal cell carcinoma, pancreatic adenocarcinoma, ovarian carcinoma and endometrial cancer.

### MUC4

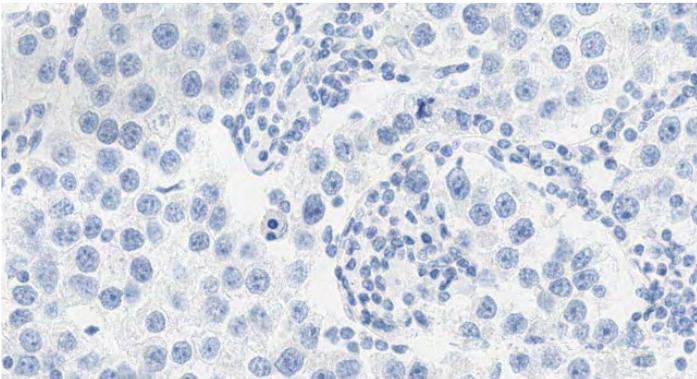
Breast fibroadenoma



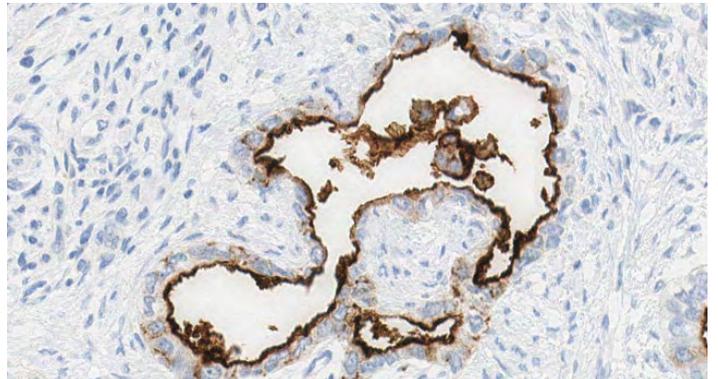
Glioblastoma



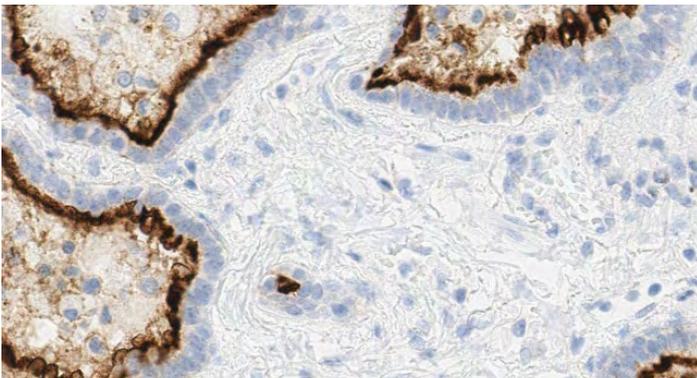
Seminoma



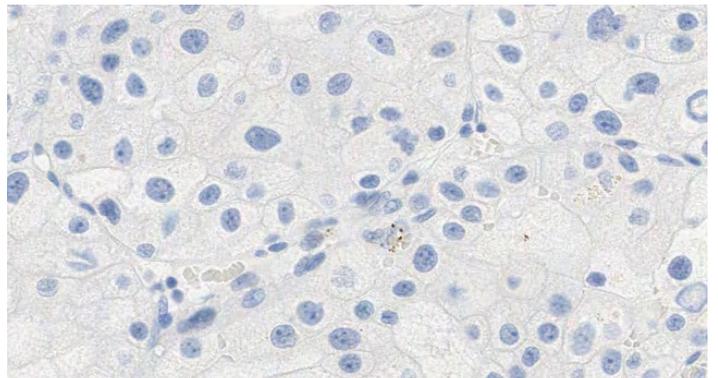
Stomach adenocarcinoma



Lung NSCLC

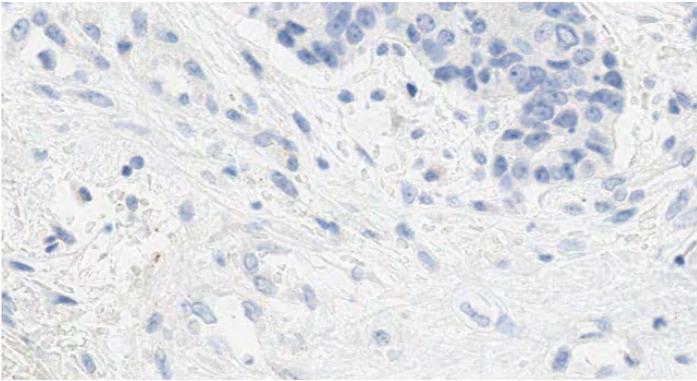


Renal cell carcinoma

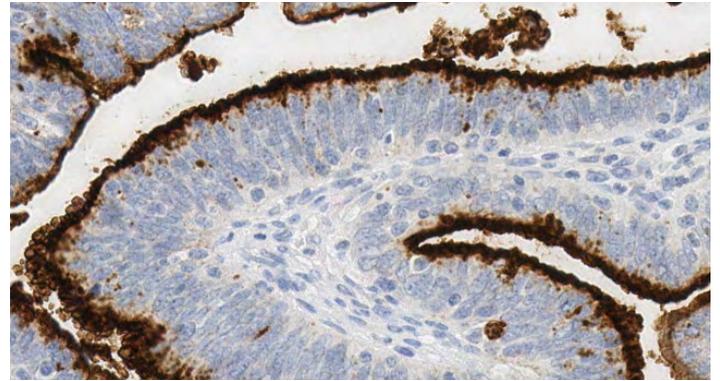


## MUC4

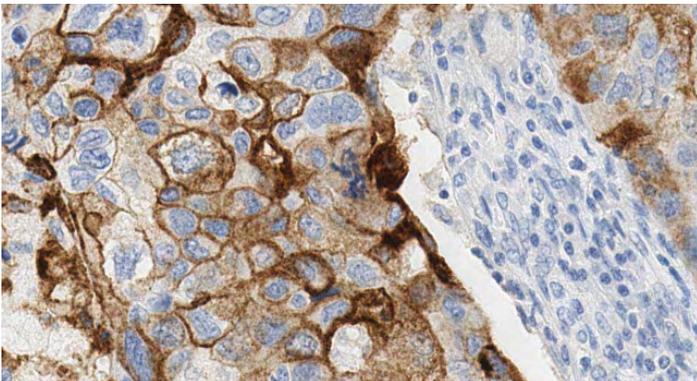
Pancreatic adenocarcinoma



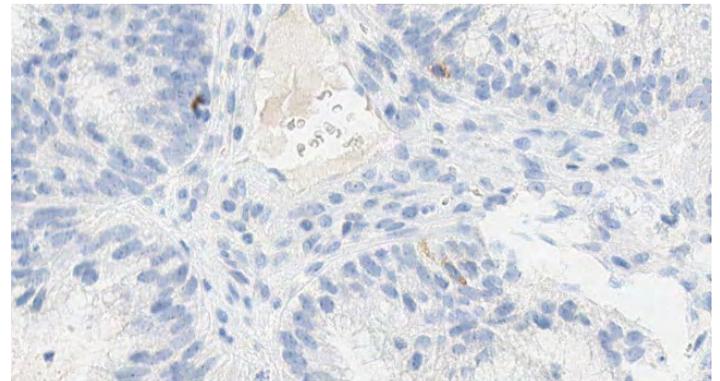
Cervical adenocarcinoma



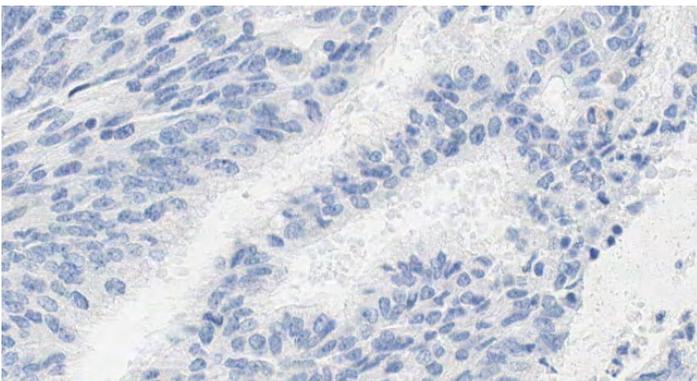
Cervical squamous cell carcinoma



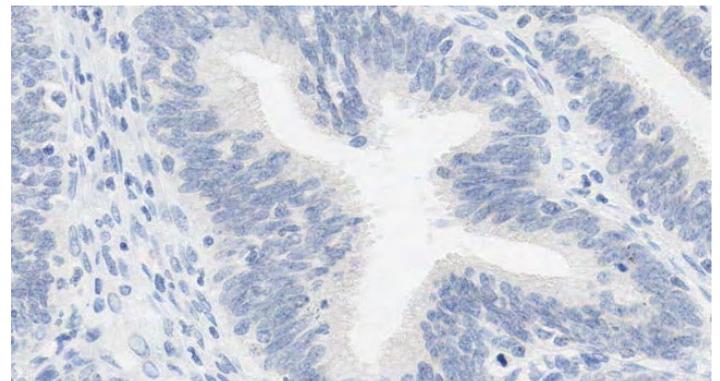
Ovarian carcinoma



Endometrial cancer



Isotype control - Cervical cancer



**Figure 2. MUC4 expression in cancer.** IHC staining of multi-cancer human tissues using anti-MUC4 (ab307546) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

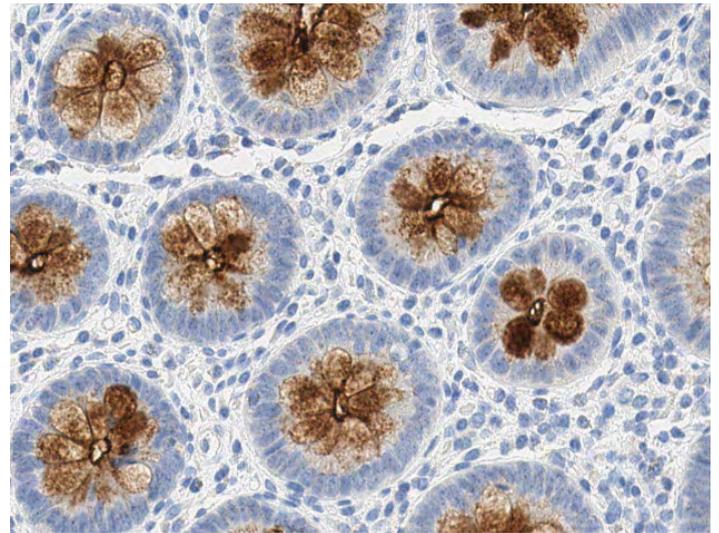
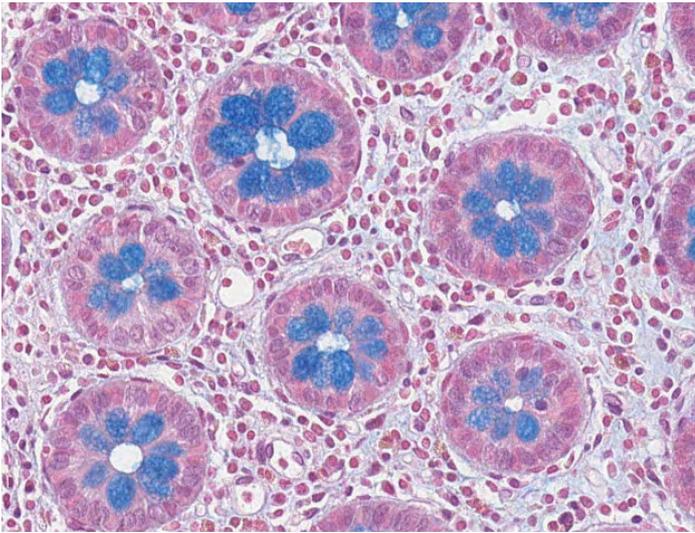
## Evaluation of mucins by alcian blue staining

Mucins found in epithelium and connective tissue can be visualized with alcian blue stain. Acidic mucins will be colored blue by alcian blue, but neutral mucins will remain uncolored. Below are the representative images of alcian blue staining and MUC4 antibody staining.

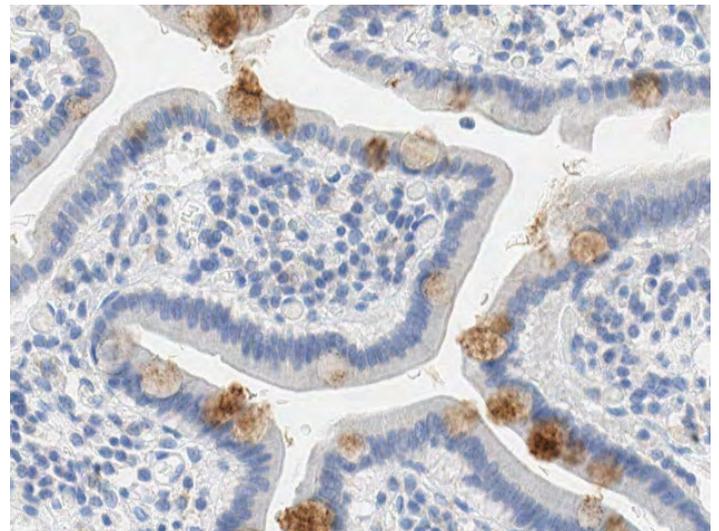
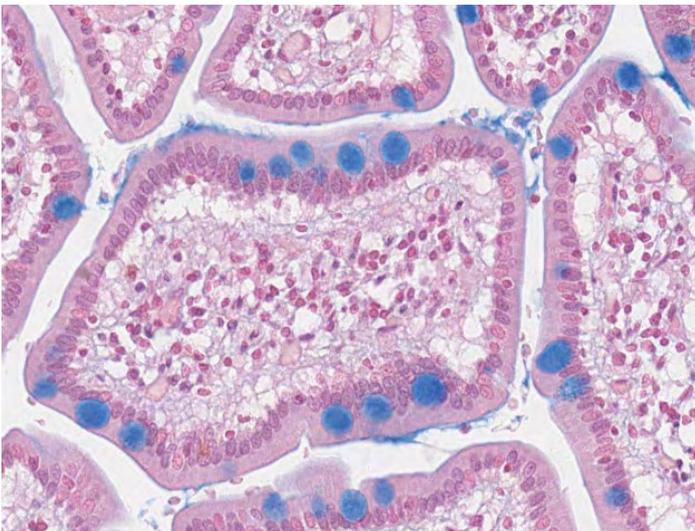
### Alcian blue

### MUC4

#### Small intestine



#### Colon



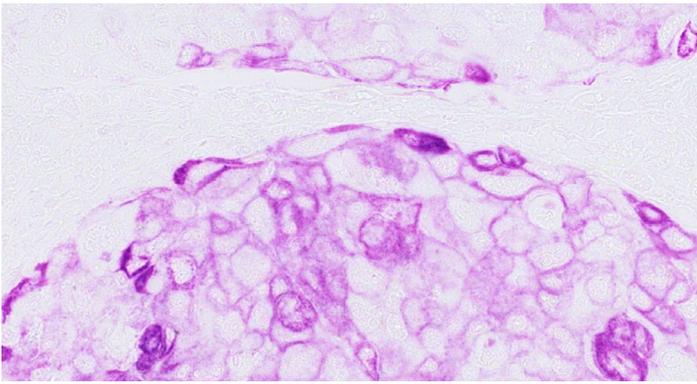
**Figure 3. Mucins in small intestine and colon.** Alcian blue staining and IHC staining of normal human tissues using alcian blue stain kit (ab150662) and anti-MUC4 (ab307546) antibody. Positive staining in blue (a & c) and brown (b & d); nuclear fast red counterstain in red (a & c), nuclear hematoxylin counterstain in blue (b & d). Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## MUC4 expression in cervical cancer (DISCOVERY ULTRA)

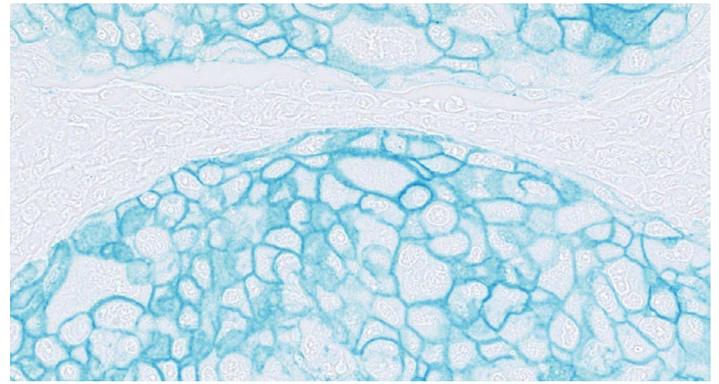
MUC4 is expected to colocalize with HER2 at the cell surface and in the cytoplasm, where it activates HER2 signaling. Below are representative images of MUC4 and HER2 in two individual cases of cervical cancer, imaged using a duplex chromogenic assay.

### Cervical squamous cell carcinoma

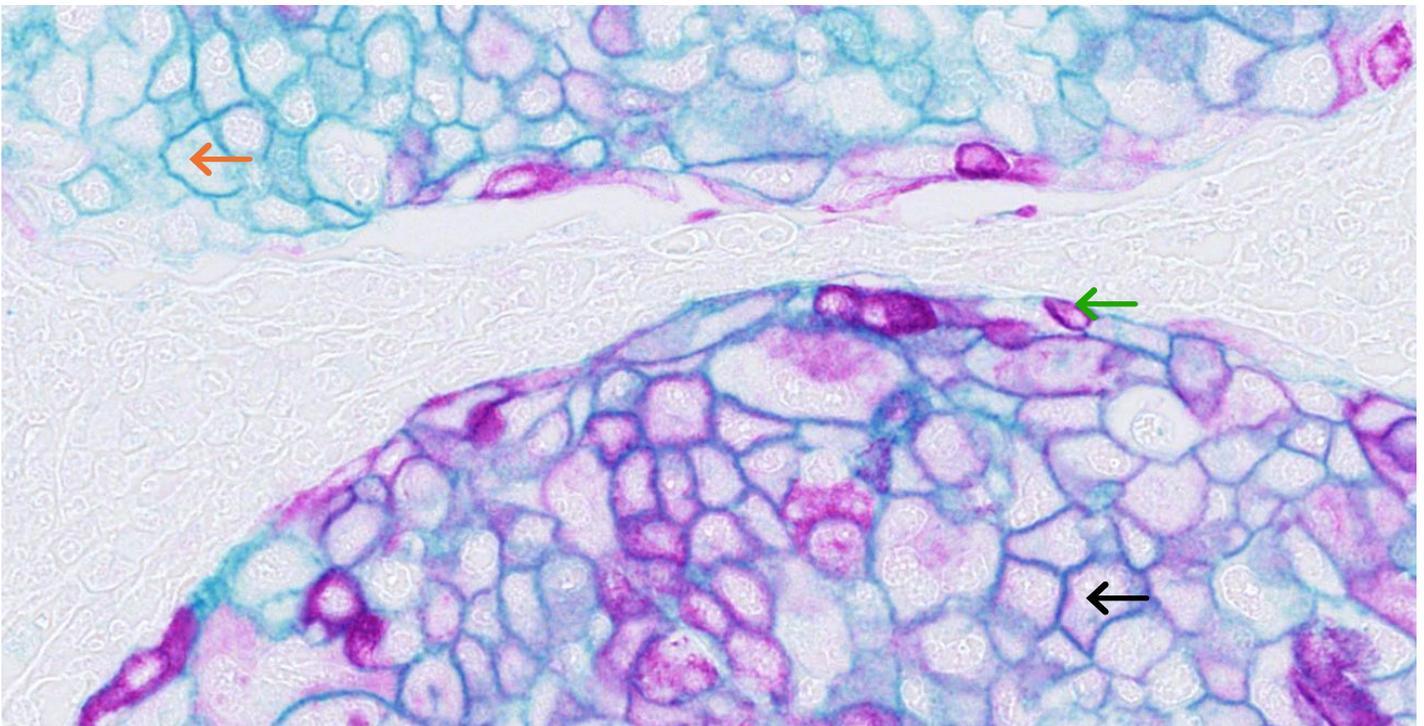
MUC4 (a)



HER2 (b)

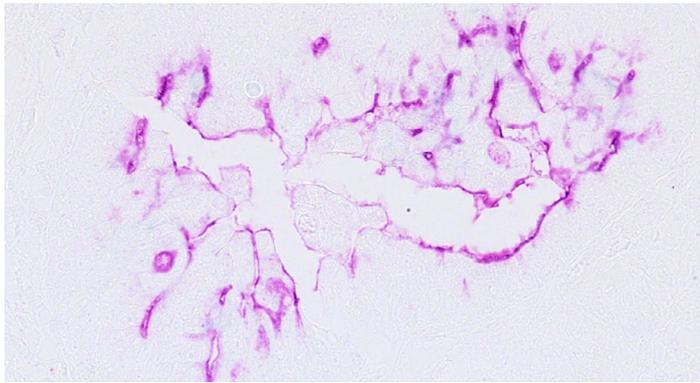


MUC4 / HER2 / HER2 & MUC4 (c)

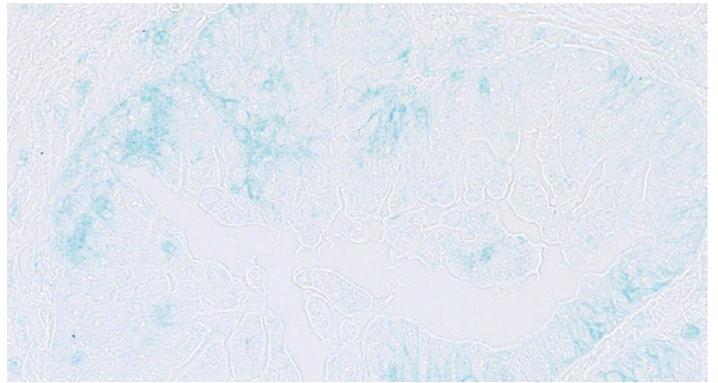


Enhanced validation data

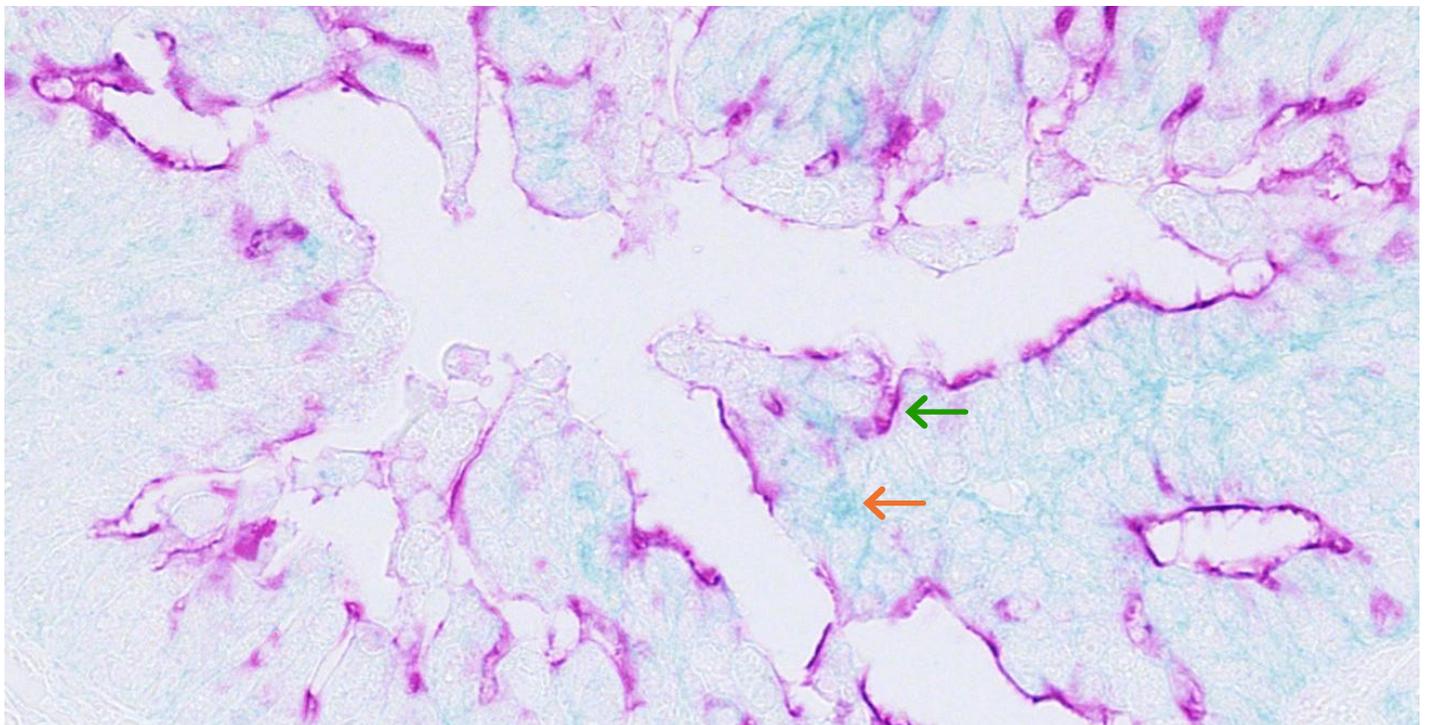
MUC4 (d)



HER2 (e)



MUC4 / HER2 / HER2 & MUC4 (f)



**Figure 4. MUC4 and HER2 expression using a duplex co-localization assay.** IHC staining of sequential sections of human cervical cancer tissue. The green, orange, and black arrows represent MUC4+ (purple), HER2+ (teal), and MUC4+HER2+ (blue) cells. The below table describes the staining conditions used in the duplex assay.

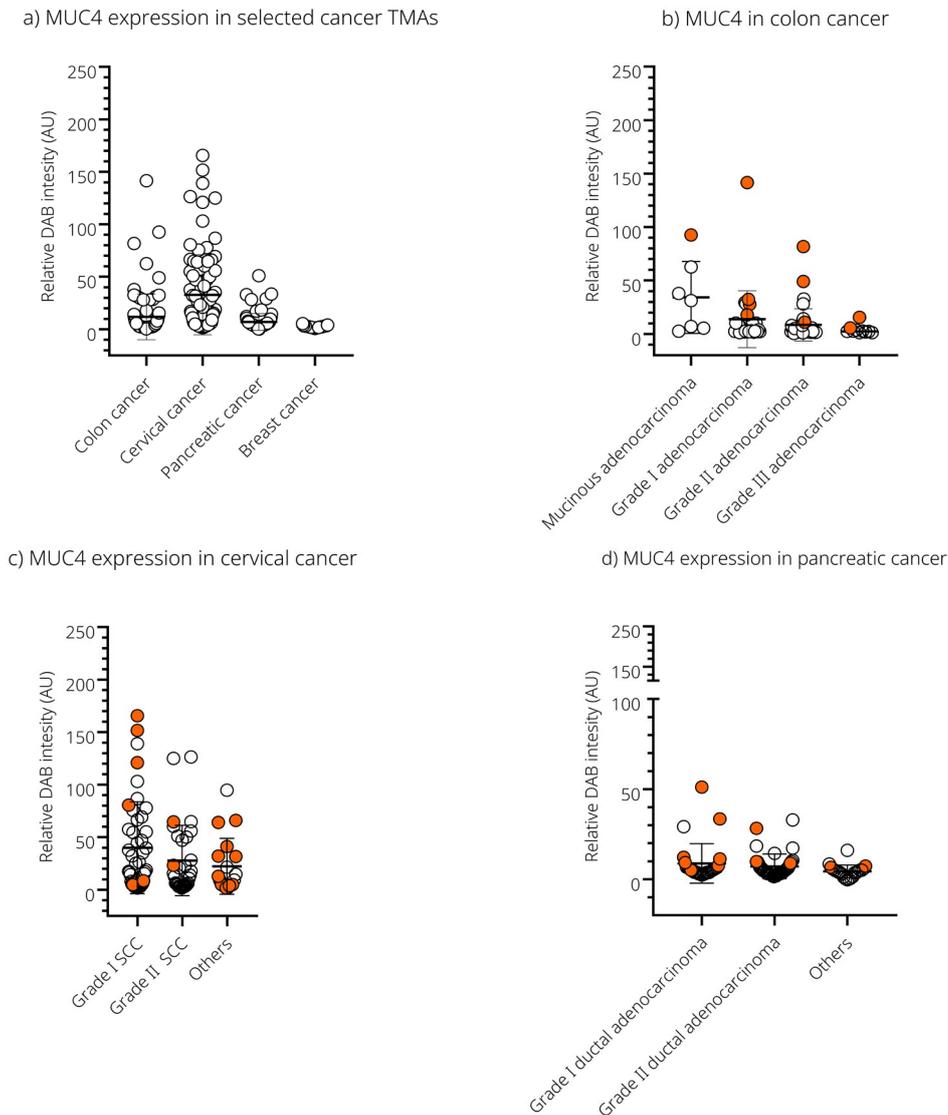
Figure	1 <sup>st</sup> Primary antibody (purple)	2 <sup>nd</sup> Primary antibody (teal)
4 a & d	MUC4	IHC diluent
4 b & e	IHC diluent	HER2
4 c & f	MUC4	HER2

Slides were scanned at 40x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 40X on Aperio® ImageScope.

NanoZoomer® is a registered trademark of Hamamatsu Photonics K.K.

## MUC4 expression in cancer (DISCOVERY ULTRA)

MUC4 expression varied in the analyzed cancer TMAs, with cervical cancer showing the highest relative DAB intensity and breast cancer the lowest. The staining intensity of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD), excluding breast cancer. The IHC images corresponding to orange data points are shown in Figures 6-8.



**Figure 5. MUC4 protein expression in a selection of cancer TMAs.**

**(a)** The scatter plot (with SD) summarizes the relative average DAB intensity of MUC4 expression in selected cancer TMA cores (colon cancer (97), cervical cancer (94), pancreatic cancer (89) and breast cancer (55))

**(b)** Relative average DAB intensity from 97 cores/cases of colon cancer ( Mucinous adenocarcinoma (7), grade I (includes grade 1 & 1.5) adenocarcinoma (29), grade II (includes grade 2 & 2.5)) adenocarcinoma (41) and grade III ) adenocarcinoma (10).

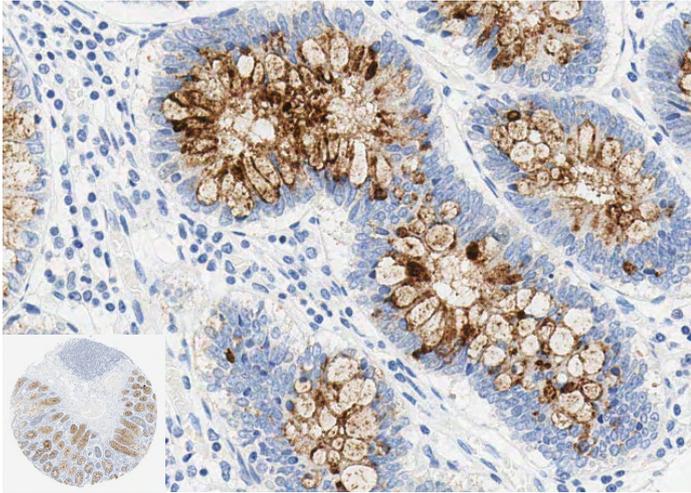
**(c)** Relative average DAB intensity from 94 cores/cases of cervical cancer squamous cell carcinoma (SCC) ( grade I (includes grade 1 & 1.5) (44) and grade II (includes grade 2 & 2.5) (31)), others include adenocarcinoma (12), mucinous adenocarcinoma (1), endometrioid carcinoma (1), adenosquamous carcinoma (5)).

**(d)** Relative average DAB intensity from 89 cores/cases of pancreatic cancer (grade I ductal carcinoma (includes grade 1 & 1.5) (28) and grade II ductal carcinoma (includes grade 2 & 2.5) (39)), others (22) include acinar cell carcinoma (1), mucinous cystadenocarcinoma (2), Mucinous adenocarcinoma (1), islet cell tumor (6), Neuroendocrine carcinoma (4), Papillary carcinoma (1), Solid-pseudopapillary tumor (2), Mixed acinar-endocrine carcinoma (1), Gastrointestinal stromal tumor, GIST (1), Metastatic stomach adenocarcinoma (1), Undifferentiated carcinoma (2).

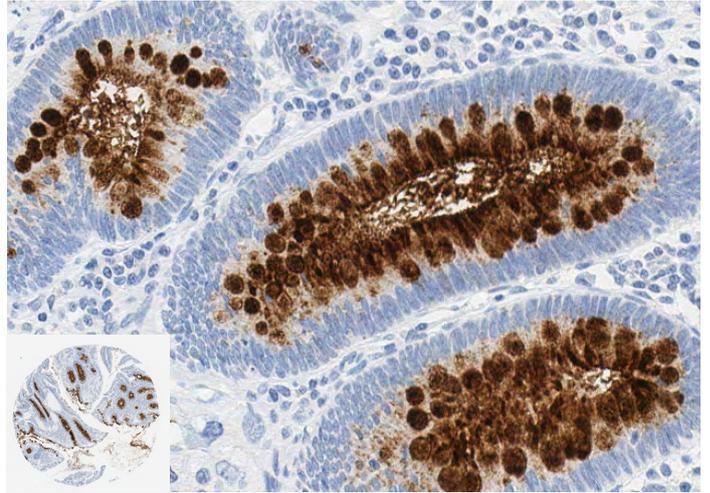
## MUC4 expression in colon cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human colon cancer TMA showing weak to strong MUC4 expression.

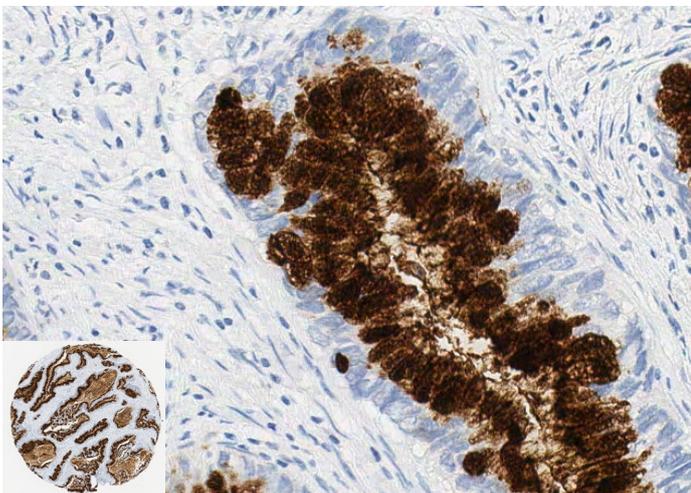
**Tubular adenoma**  
(25.25 ; 1+)



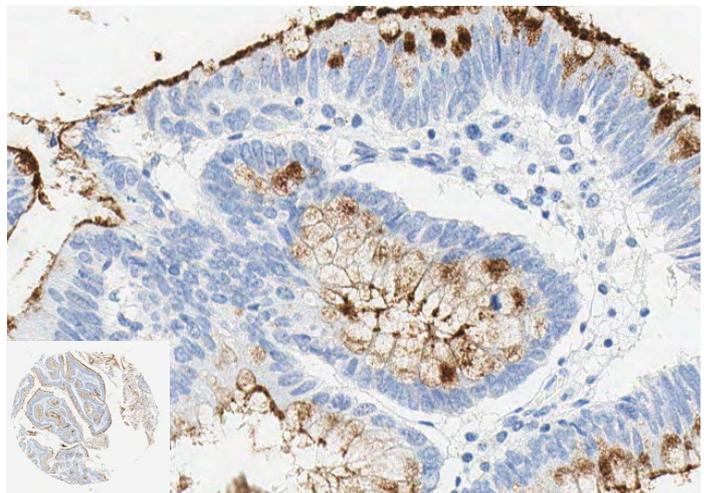
**Papillary adenocarcinoma**  
(31.32 ; 1+)



**Mucinous adenocarcinoma**  
(92.54 ; 2+)

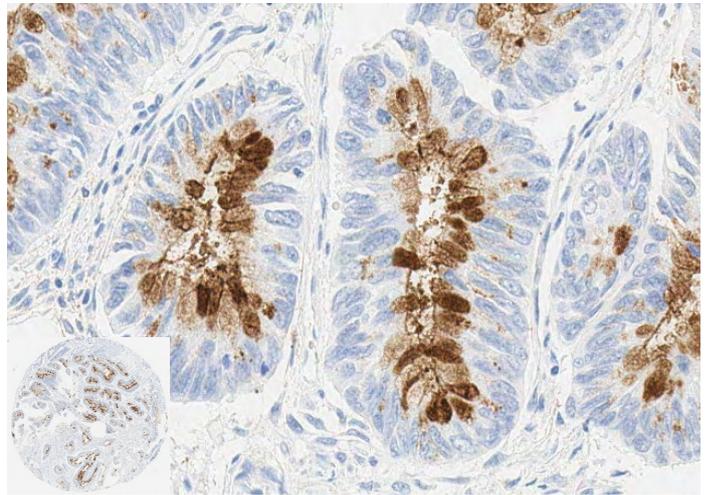
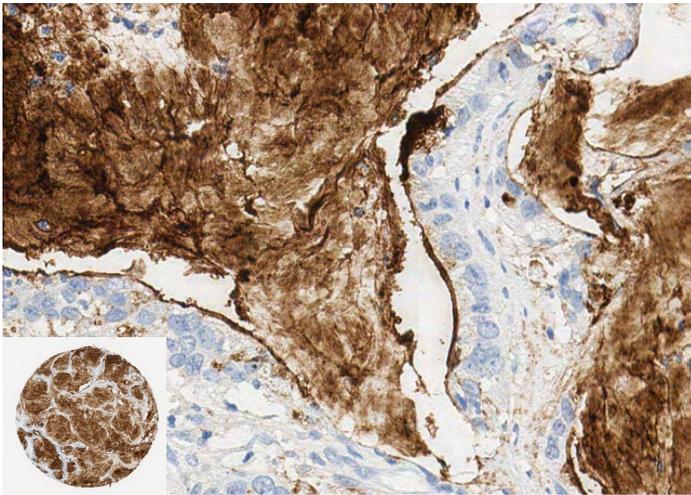


**Grade I adenocarcinoma**  
(27.84 ; 1+)



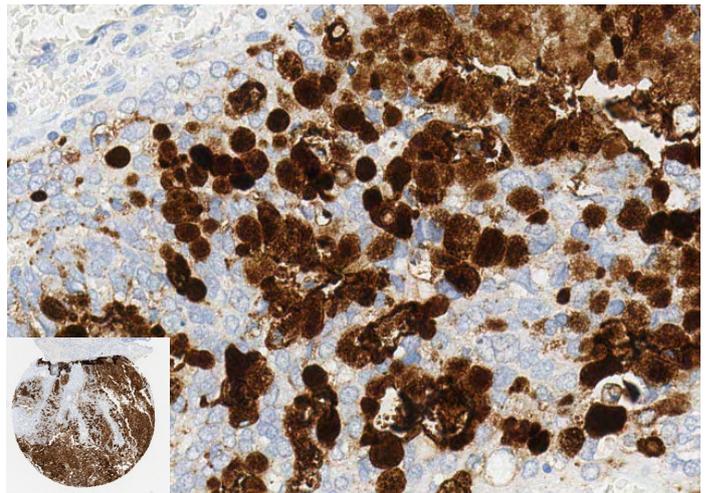
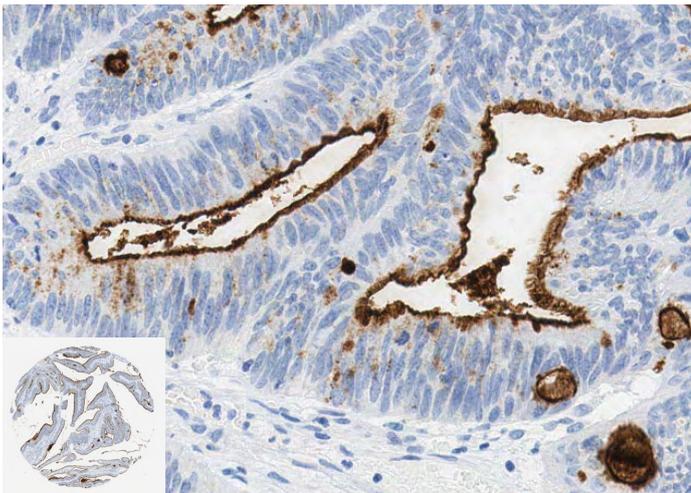
**Grade I adenocarcinoma**  
(141.62 ; 2+)

**Grade I/II adenocarcinoma**  
(17.85 ; 1+)



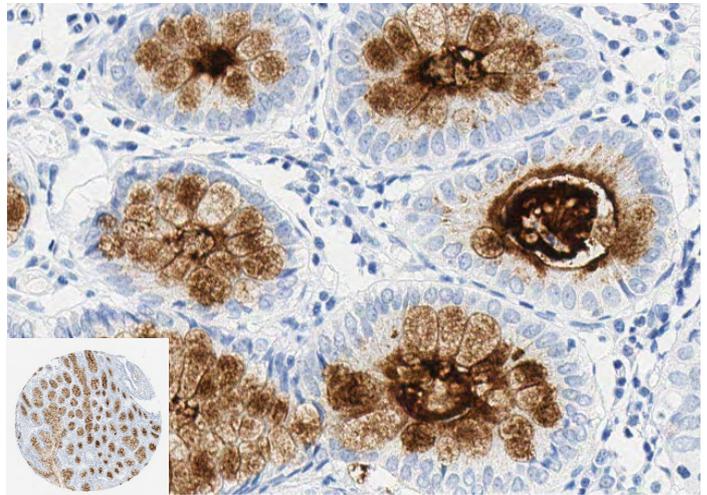
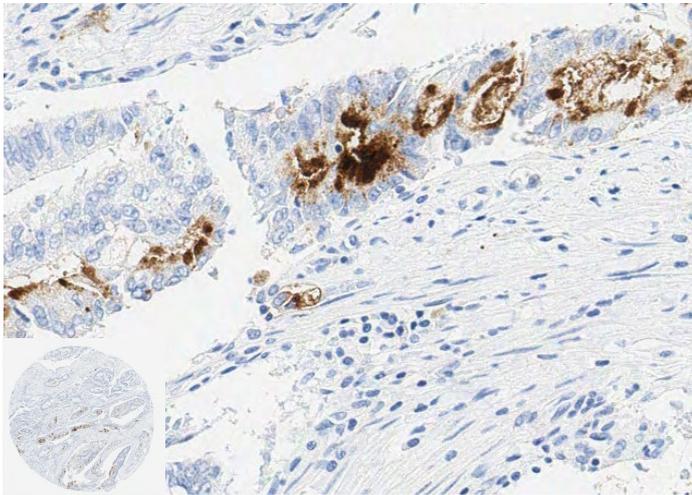
**Grade I/II adenocarcinoma**  
(32.36 ; 1+)

**Grade II adenocarcinoma**  
(10.91 ; 1+)



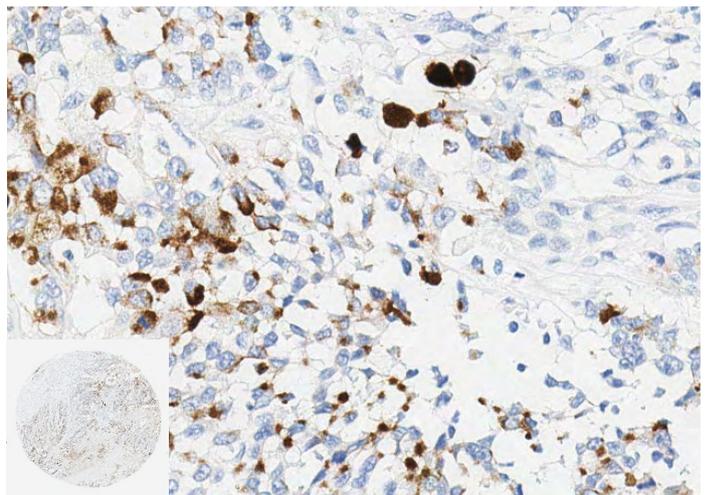
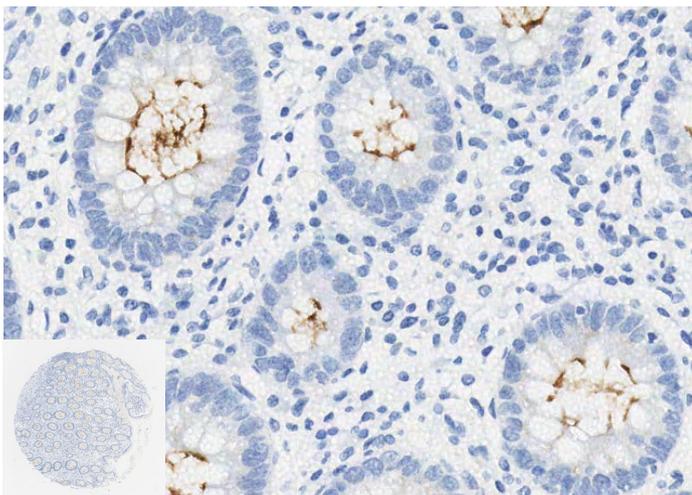
**Grade II adenocarcinoma**  
(81.75 ; 2+)

**Grade II/III adenocarcinoma**  
(49.11 ; 1+)



**Grade III adenocarcinoma**  
(5.57 ; 0)

**Grade III adenocarcinoma**  
(15.57 ; 1+)

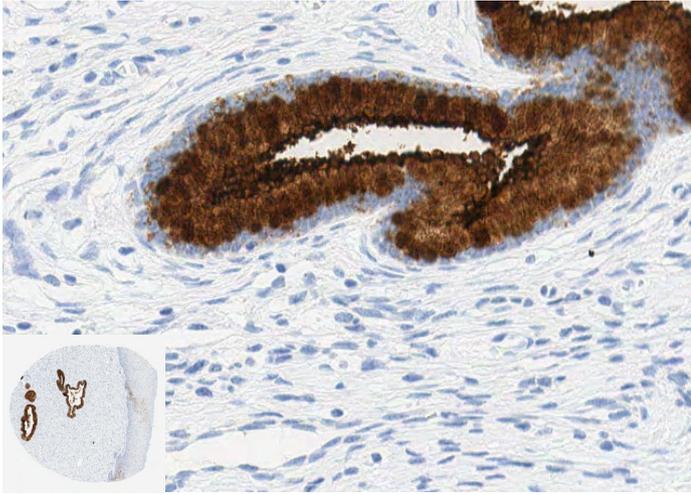


**Figure 6. MUC4 expression in colon cancer.** IHC images showing MUC4 staining with relative average DAB intensity and corresponding intensity score (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

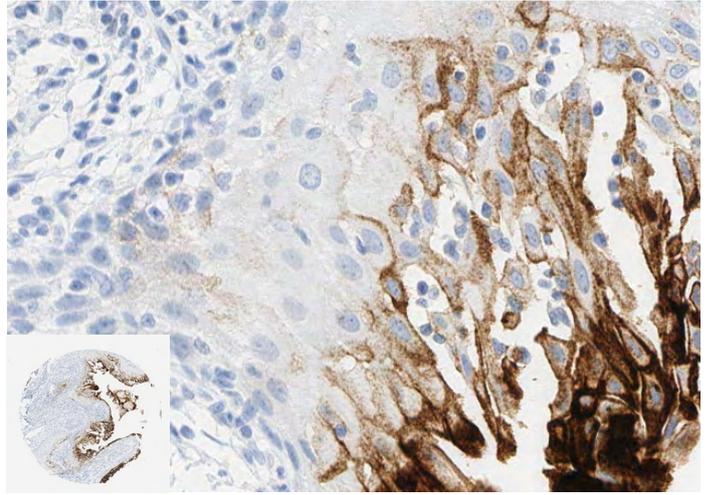
## MUC4 expression in cervical cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human cervical cancer TMA showing weak to strong MUC4 expression.

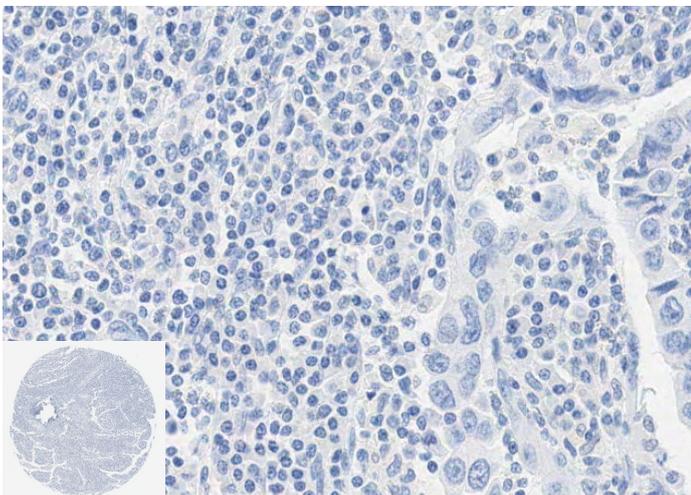
**Cervicitis**  
(14.58 ; 1+)



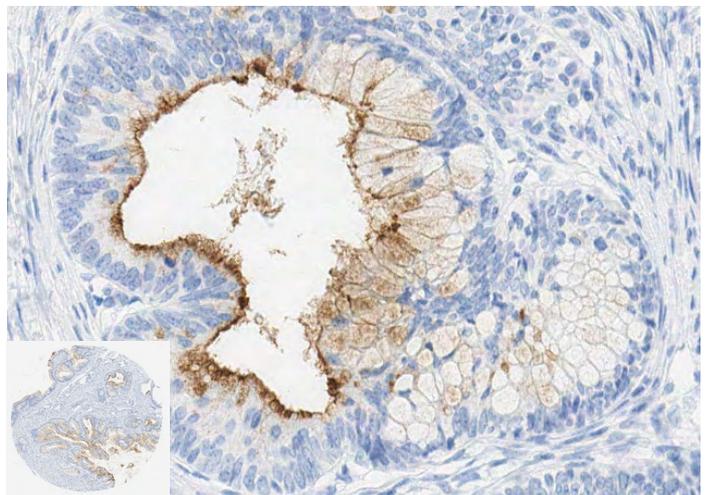
**Mucinous adenocarcinoma**  
(31.95 ; 1+)



**Grade II / III adenocarcinoma**  
(3.88 ; 0)

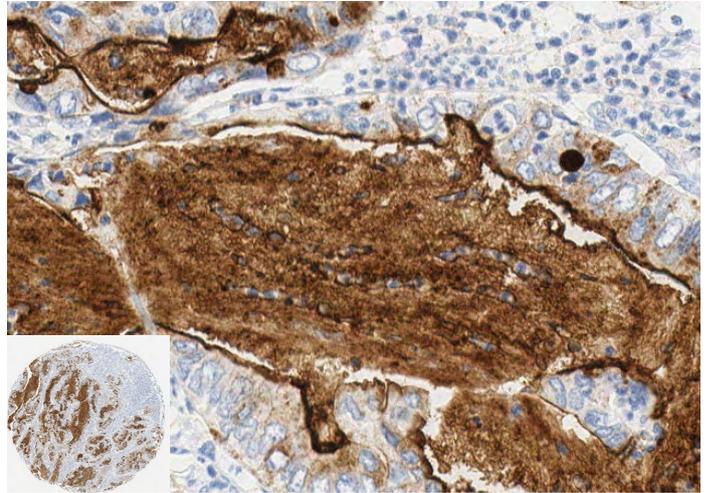
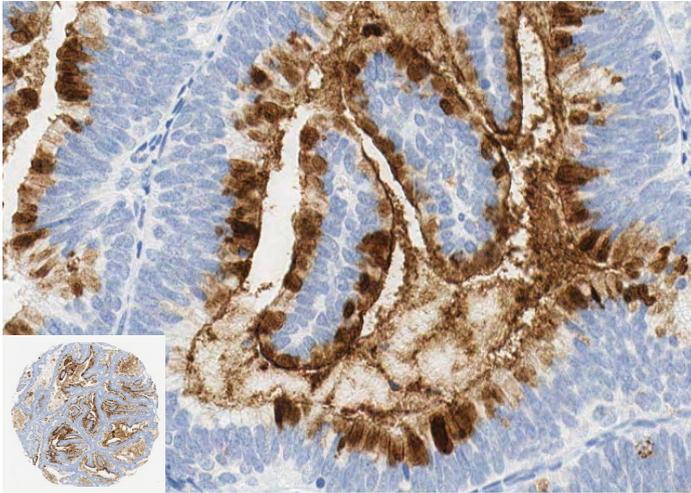


**Grade I adenocarcinoma**  
(12.82 ; 1+)



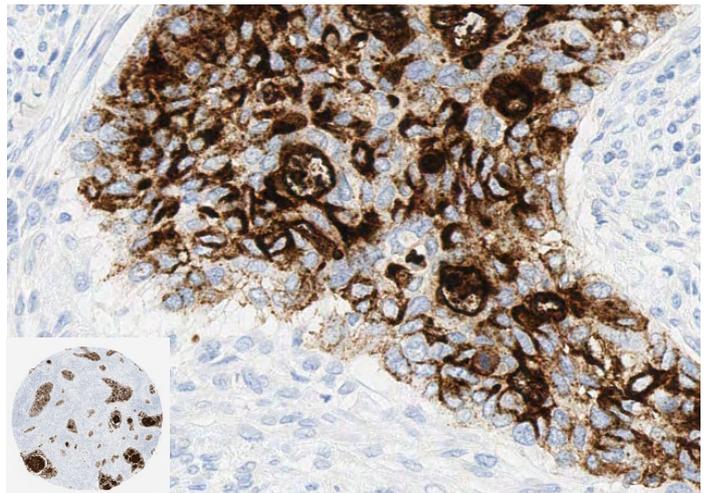
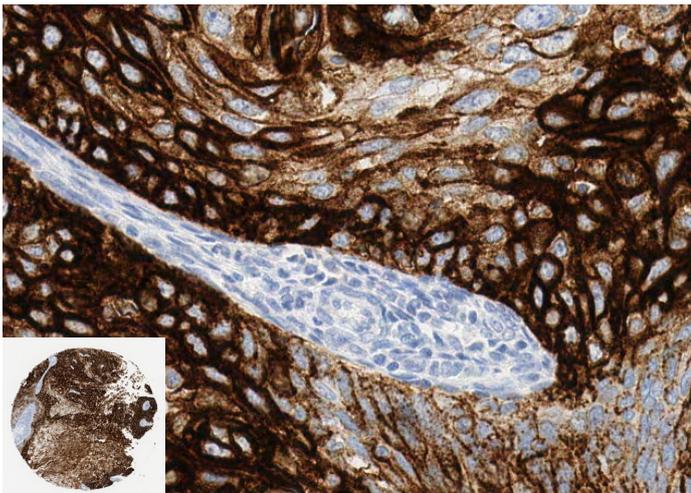
**Grade II adenocarcinoma**  
(41.05 ; 1+)

**Grade II adenocarcinoma**  
(64.72 ; 1+)



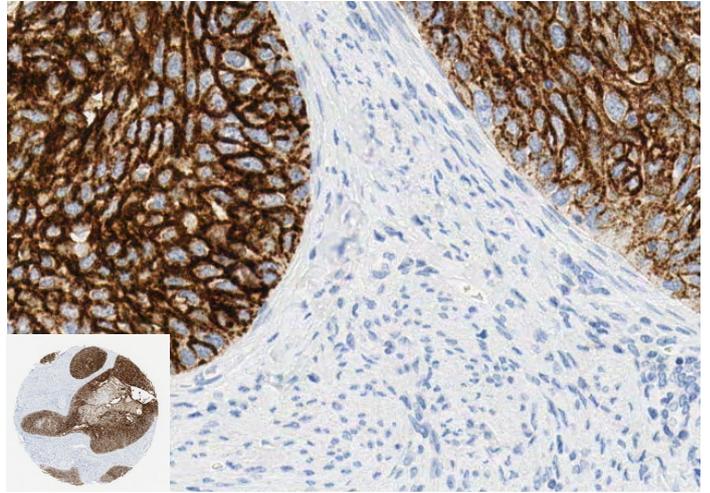
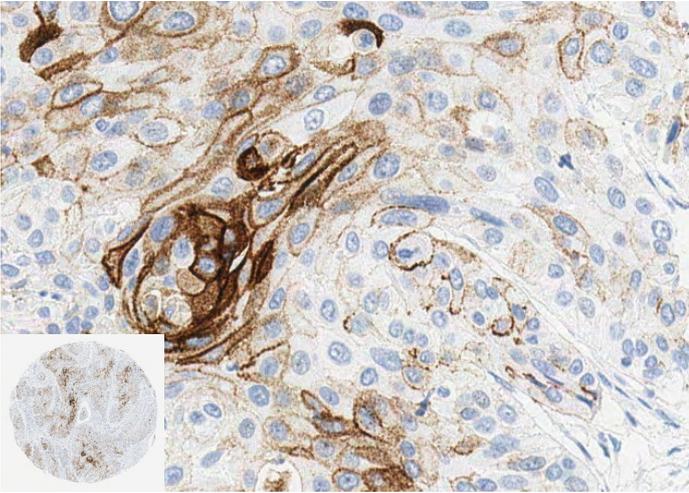
**Grade I / II squamous cell carcinoma**  
(165.55 ; 3+)

**Grade II / III squamous cell carcinoma**  
(31.56 ; 1+)



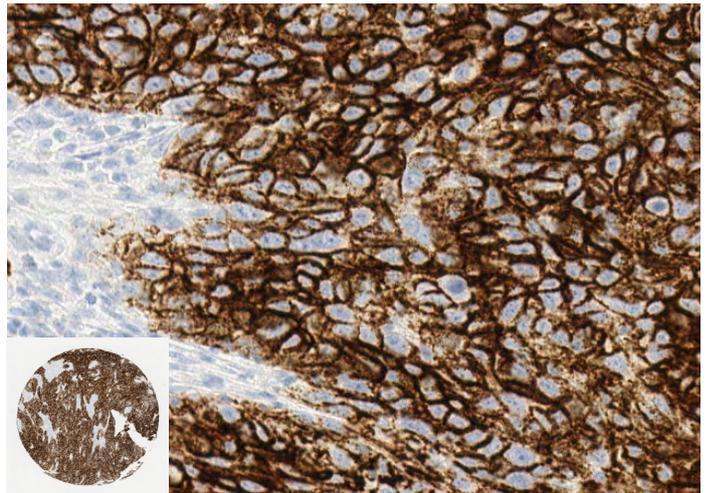
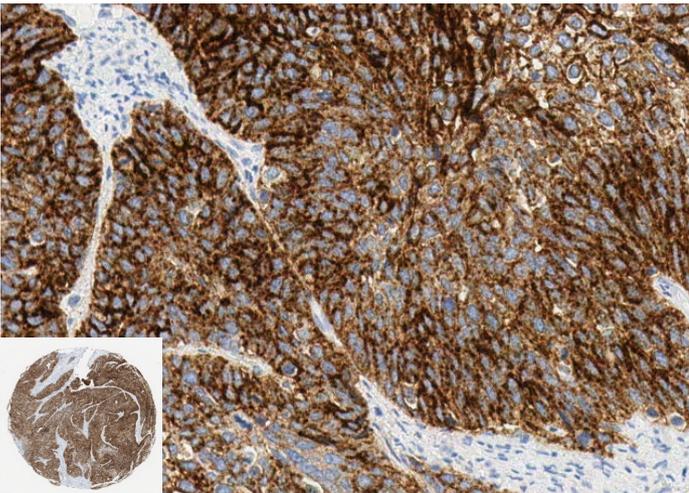
**Grade II / III squamous cell carcinoma**  
(23.38 ; 1+)

**Grade III squamous cell carcinoma**  
(80.63 ; 2+)



**Grade III squamous cell carcinoma**  
(120.98 ; 2+)

**Grade III squamous cell carcinoma**  
(151.60 ; 2+)

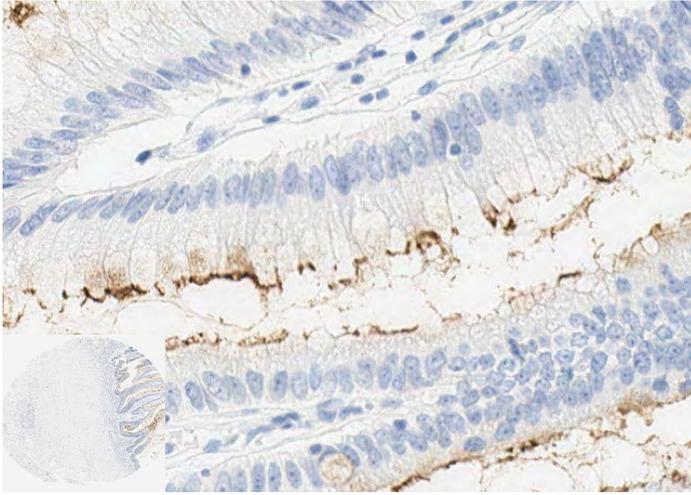


**Figure 7. MUC4 expression in cervical cancer.** IHC images showing MUC4 staining with relative average DAB intensity and corresponding intensity score (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

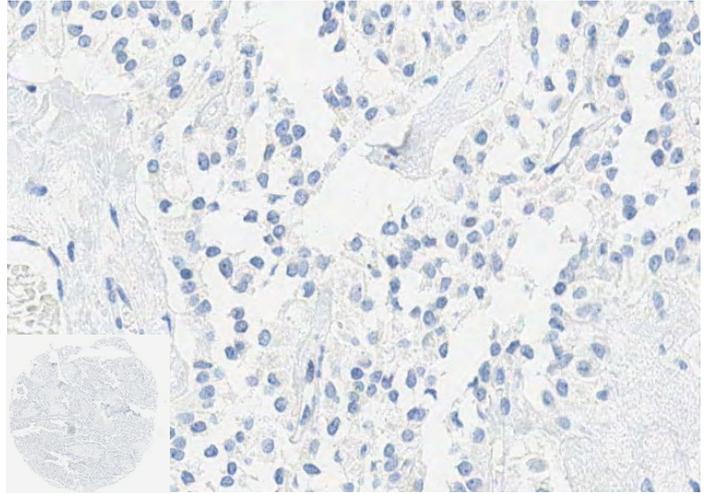
## MUC4 expression in pancreatic cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human pancreatic cancer TMA showing weak to strong MUC4 expression.

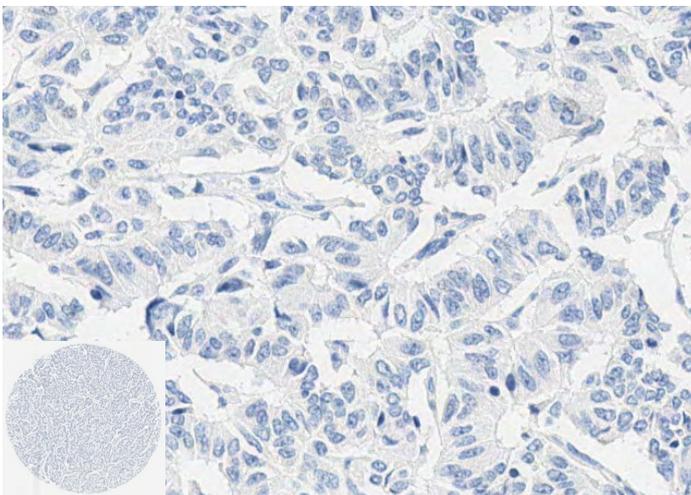
**Mucinous cystadenocarcinoma**  
(Positive staining in epithelial lining)



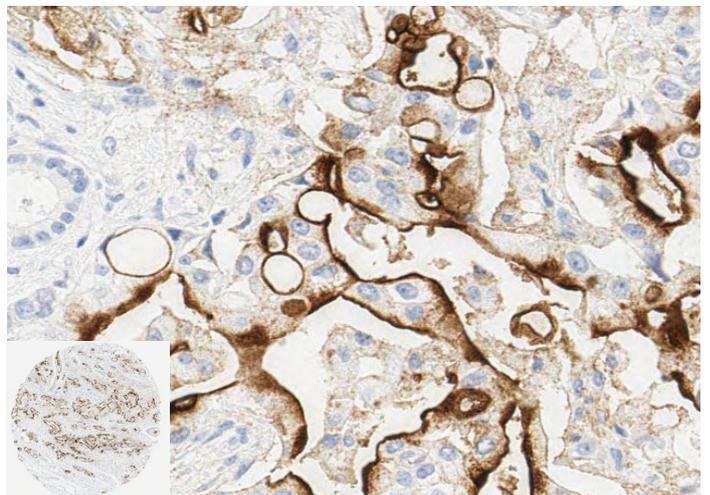
**Islet cell tumor**  
(4.45 ; 0)



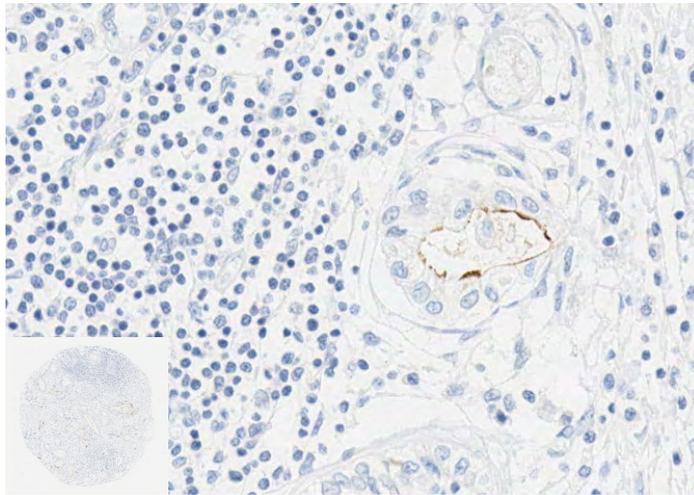
**Neuroendocrine carcinoma**  
(4.17 ; 0)



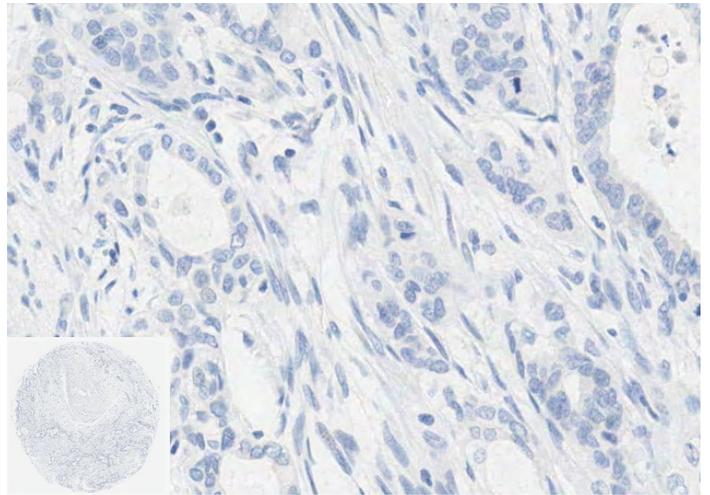
**Grade I ductal adenocarcinoma**  
(33.46 ; 1+)



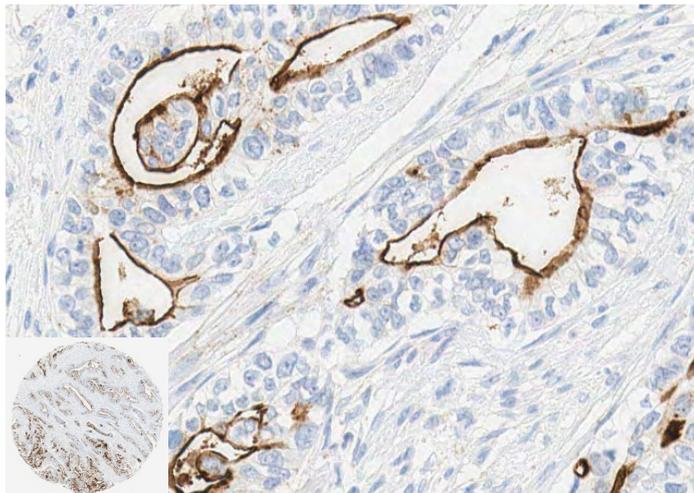
**Grade I ductal adenocarcinoma**  
(4.50 ; 0)



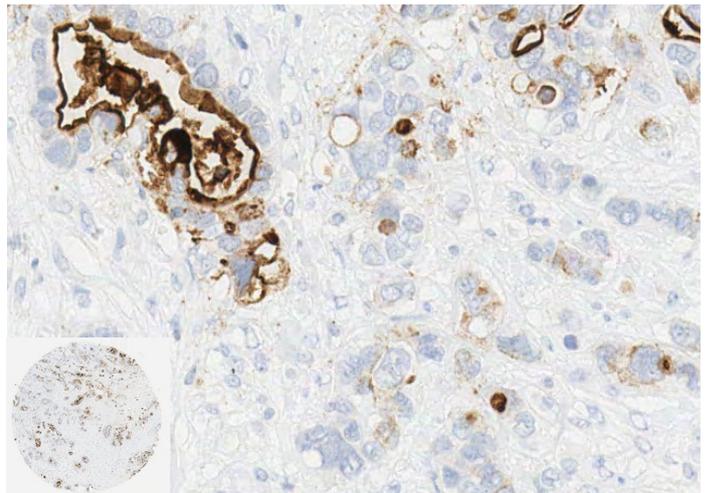
**Grade II ductal adenocarcinoma**  
(3.00 ; 0)



**Grade II ductal adenocarcinoma**  
(28.23 ; 1+)

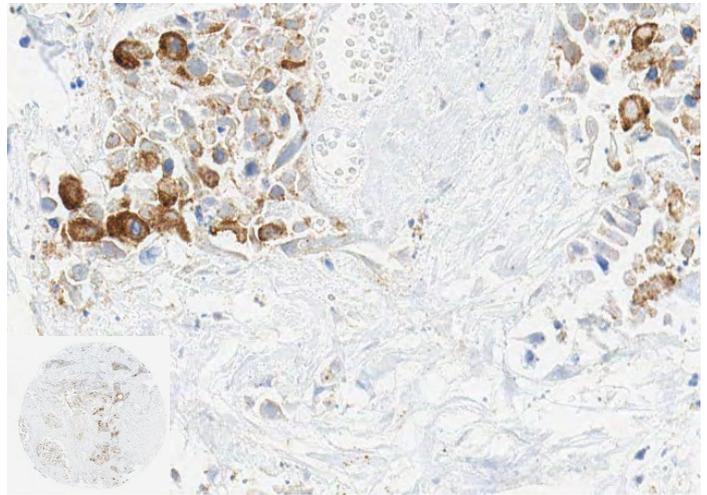
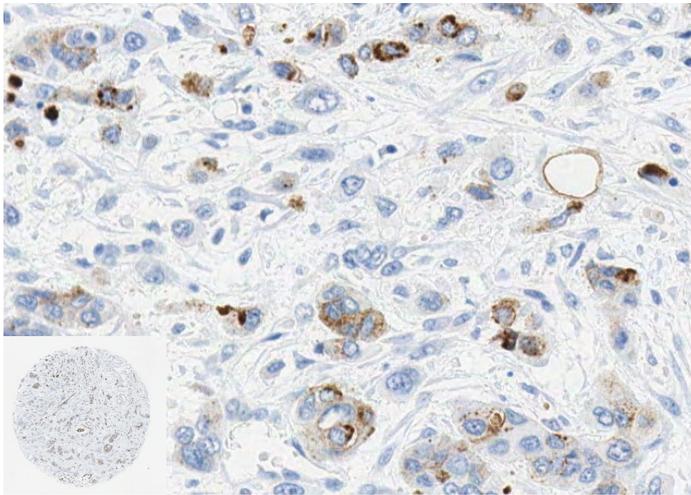


**Grade II / III ductal adenocarcinoma**  
(17.33 ; 1+)



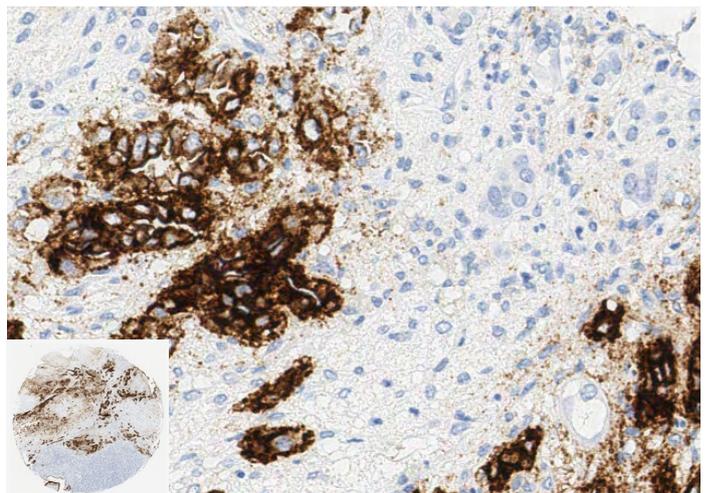
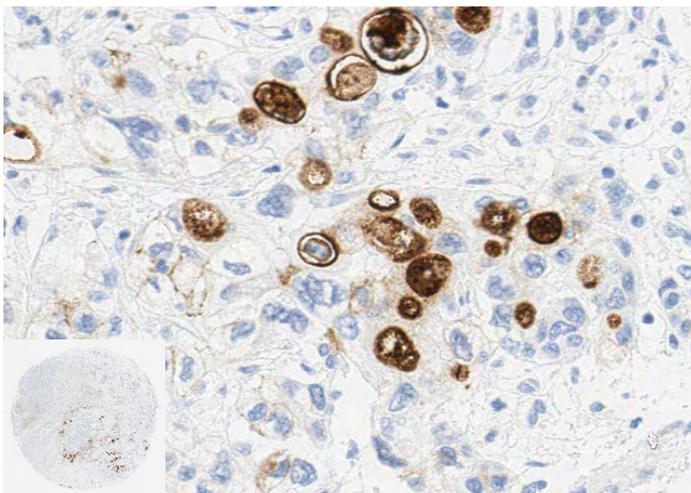
**Grade III ductal adenocarcinoma**  
(9.25 ; 0)

**Grade III ductal adenocarcinoma**  
(11.25 ; 1+)



**Grade III ductal adenocarcinoma**  
(12.17 ; 1+)

**Grade III ductal adenocarcinoma**  
(51.12 ; 1+)

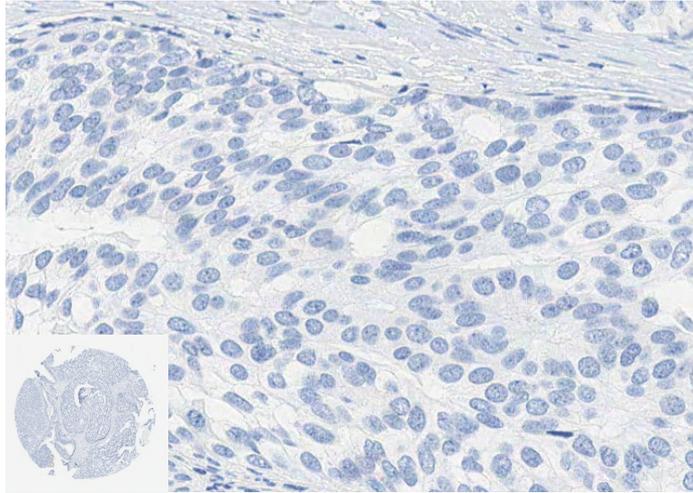


**Figure 8. MUC4 expression in pancreatic cancer.** IHC images showing MUC4 staining with relative average DAB intensity and corresponding intensity score (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

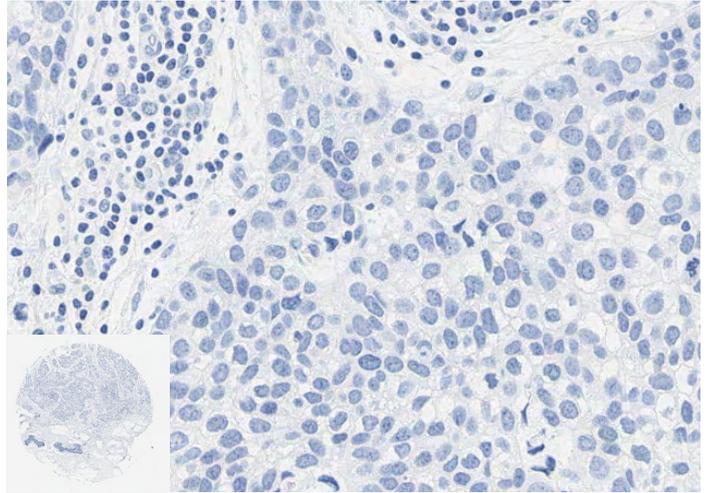
## MUC4 expression in breast cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human breast cancer TMA showing no MUC4 expression.

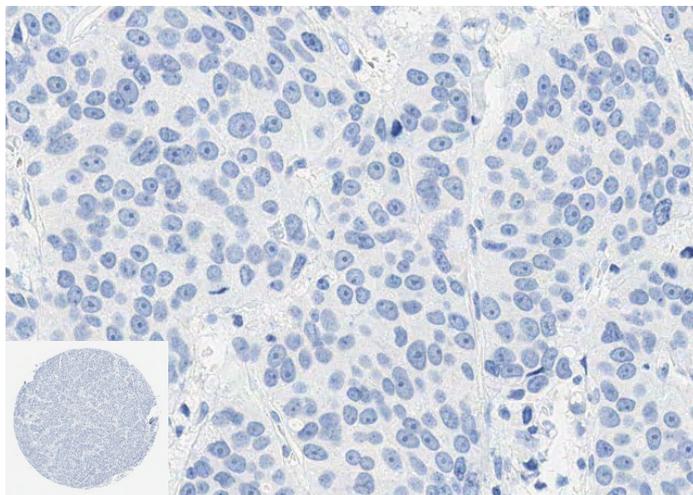
**Grade I ductal carcinoma *in situ***  
(1.42 ; 0)



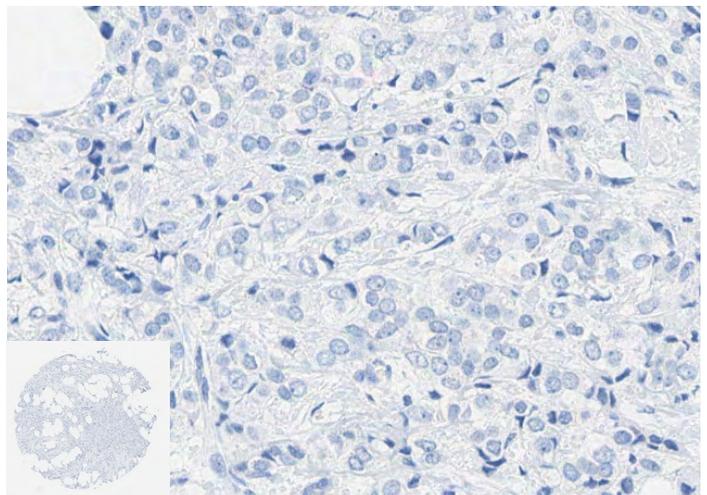
**Grade I ductal carcinoma *in situ***  
(1.77 ; 0)



**Grade II / III invasive ductal carcinoma**  
(2.23 ; 0)



**Grade III invasive ductal carcinoma**  
(1.13 ; 0)



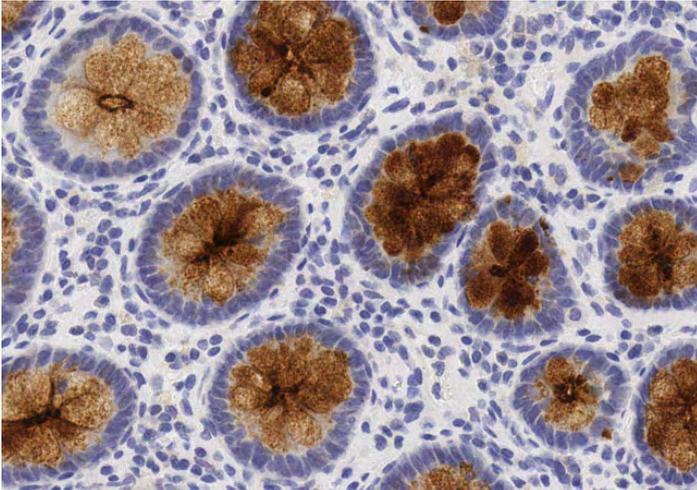
**Figure 9. MUC4 expression in breast cancer.** IHC images showing MUC4 staining with relative average DAB intensity and corresponding intensity score (a-l). Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## MUC4 expression in normal tissues (BOND™ RX)

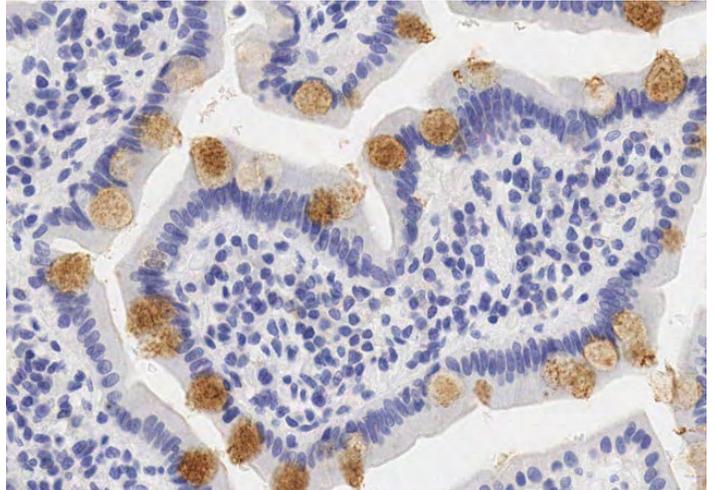
Below are the representative images of small intestine and colon tissues showing MUC4 expression.

### MUC4

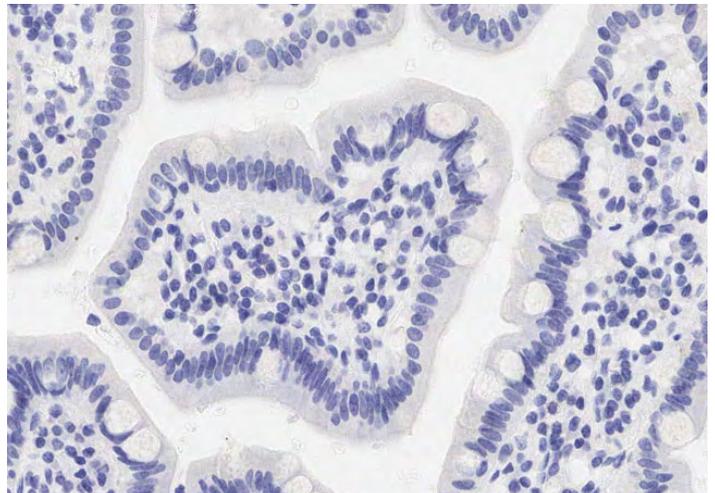
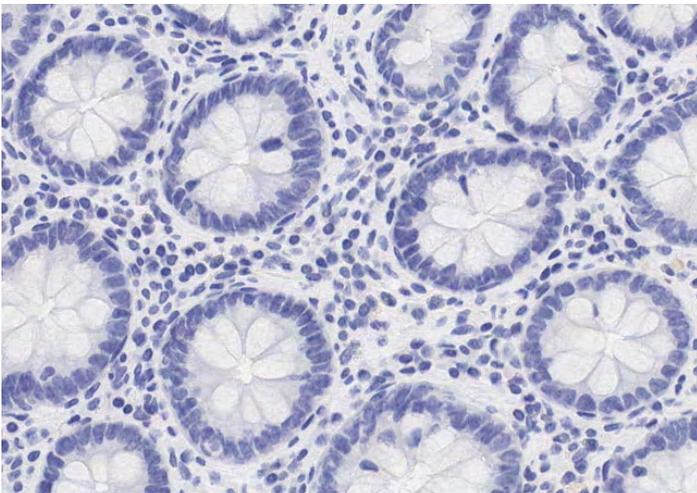
Small intestine



Colon



### Isotype control



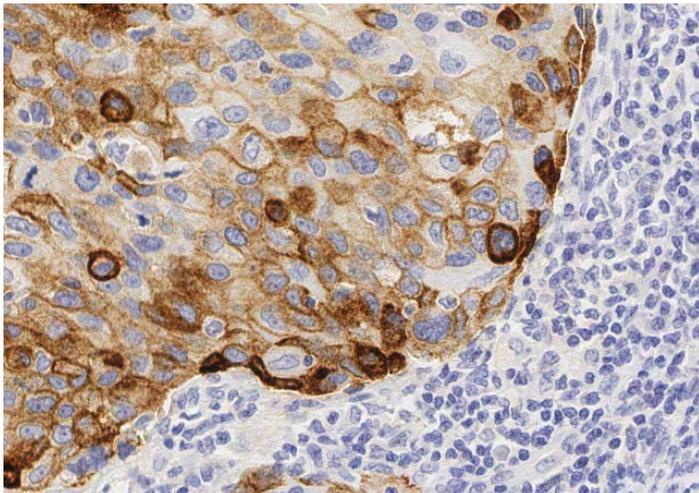
**Figure 10. MUC4 expression in human normal tissue.** IHC staining of human small intestine and colon tissues using anti-MUC4 (ab307546) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; Hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## MUC4 expression in cancer tissues (BOND™ RX)

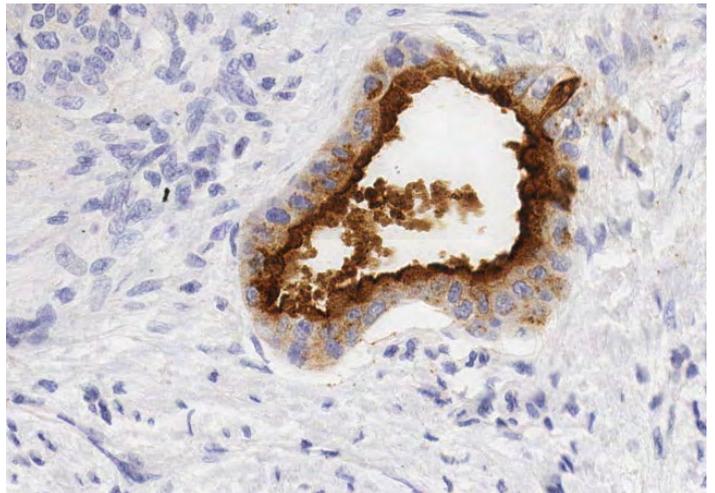
Below are the representative images of cervical cancer and stomach adenocarcinoma showing MUC4 expression.

### MUC4

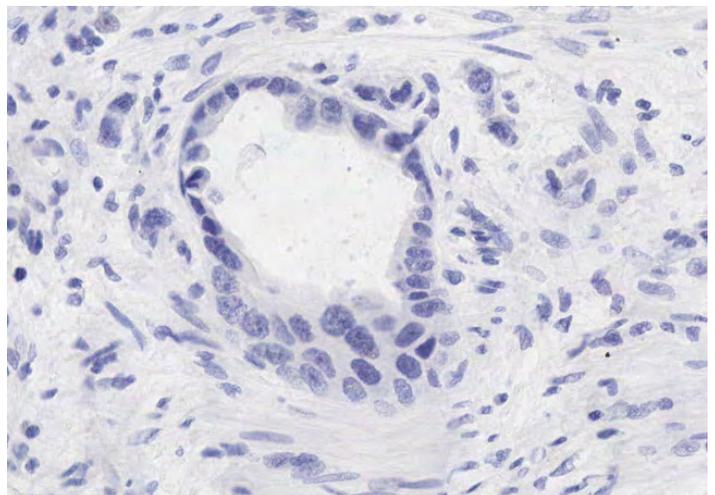
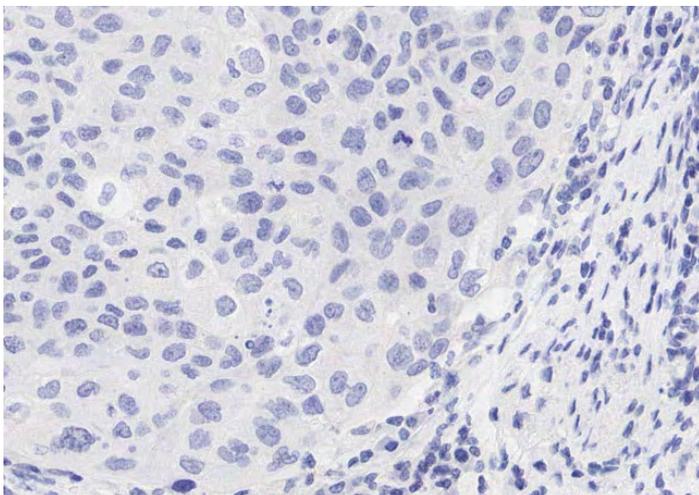
Cervical cancer



Stomach adenocarcinoma



Isotype control



**Figure 11. MUC4 expression in human cancer tissue.** IHC staining of human cervical cancer and stomach adenocarcinoma tissues using anti-MUC4 (ab307546) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; Hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

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## Enhanced validation data

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