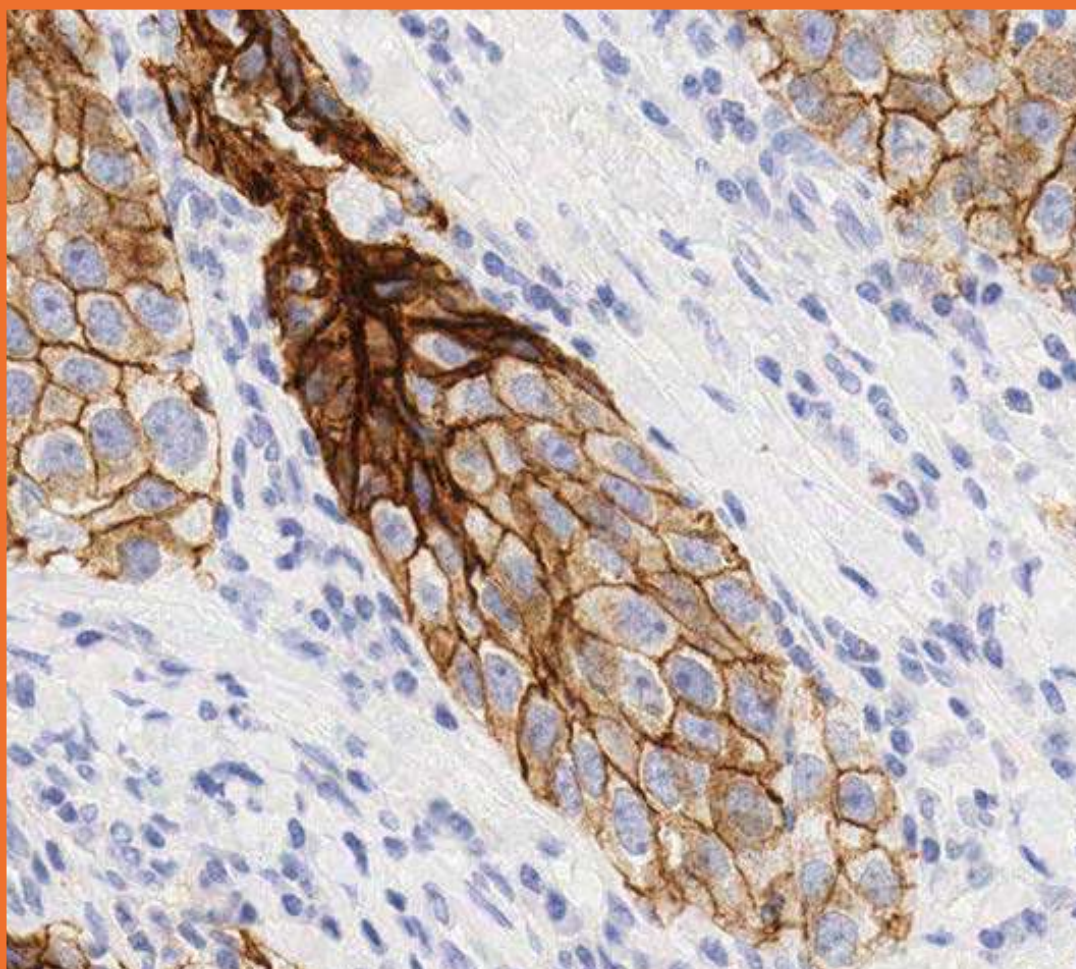


Enhanced validation data

Anti-Claudin 6 recombinant antibody – ab314134



Enhanced validation of Anti-Claudin 6 recombinant antibody [EPR28103-113] – ab314134

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 immuno-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-Claudin 6 recombinant antibody ab314134 in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®) and DISCOVERY ULTRA system (Roche Diagnostics).

Target overview

HGNC symbol

CLDN6

Approved name

Claudin 6

Chromosomal location

16p13.3

Function

- Functions as a tight junction molecule, enabling cell-to-cell adhesion in epithelial or endothelial cells.

Tissue specificity

- Expressed in embryonic stem cells committed to an epithelial fate during development. CLDN6 is undetectable in healthy adult tissue, unlike other CLDNs⁽¹⁻⁵⁾.
- High CLDN6 expression has been observed in solid tumors, including ovarian, lung, endometrial, and gastric cancers as well as testicular cancer and teratomas⁽⁶⁻⁸⁾.
- Claudin proteins are highly similar in their sequences. The CLDN6 protein exhibits close sequence relationships with other claudin proteins, such as CLDN3, CLDN4, CLDN5, and CLDN9.

Cellular localization

- Cell membrane. Expressed at the cell surface.

Target information above from: UniProt accession P56747

The UniProt Consortium

The Universal Protein Resource (UniProt) in 2010

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

Materials and methods

Anatomic site	Disease
Testis	Seminoma
Uterus	Endometrial cancer

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

Tissue microarray (TMA)	Cores	Cases	Normal/ Benign cases	Cancer cases	Source (#catalog number)
Multi-normal	34	34	34	0	Pantomics (#MNO 341)
Ovarian cancer	102	102	5	97	Pantomics (#OVC1021)
Testicular cancer	102	102	18	84	Pantomics (#TET1021)
Lung cancer	102	102	5	97	Pantomics (#LUC1022)

Table 2. List of human TMAs used in the enhanced validation. All tissues were sourced from Abcam-approved tissue suppliers.

Step	Reagents	Method
Deparaffinization	EZ Prep	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC2)	64 min, 100 °C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Anti-Claudin 6 recombinant antibody – [EPR28103-113] – ab314134 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 2.0 µg/mL	16 min, 37 °C
Counterstain	Hematoxylin II	8 min
Post counterstain	Bluing Reagent	4 min

Table 3. IHC staining protocol on the DISCOVERY ULTRA (Roche Diagnostics) instrument. Staining was performed using standard conditions with OptiView DAB IHC Detection kit (#760-700).

Step	Reagents	Pre-programmed protocol
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

Table 4a. IHC prestaining protocol on BOND™ RX Research Stainer (Leica®).

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-Claudin 6 recombinant antibody – [EPR28103-113] – ab314134 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 2.0 µg/mL	-	15
Wash	Bond™ wash solution	3x	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
Wash	Deionized water	1x	0
	Bond™ wash solution	1x	0
	Deionized water	1x	0

Table 4b. 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.

H-score analysis

A quantitative H-score analysis of CLDN6 expression was performed using the artificial intelligence (AI) driven digital image analysis software Visiopharm® (Version: 2023.09). TMA slides were de-arrayed and segmented into tumor and non-tumor using a trained AI model with DeepLabv3+ architecture. The segmentation performance was assessed by eye and adjusted where necessary.

Total cell numbers for each core were counted using a trained AI model with U-Net architecture. Using the cell analysis data and thresholds, H-scores of the tumor compartment for each core in the TMAs were calculated in Visiopharm® and the graphical representation was generated using the GraphPad Prism 9.

IHC staining	Corresponding intensity score	Visiopharm® intensity threshold
Negative	0	> 295
Weak	1+	< 295
Moderate	2+	< 160
Strong	3+	< 80

Table 5. Intensity scoring and thresholds for H-score analysis. The H-score captures both the IHC staining intensity and the percentage of stained cells at each intensity level. It was calculated using the formula $H\text{-score} = [(0 \times \% \text{ of negative cells}) + (1 \times \% \text{ of weak stained cells}) + (2 \times \% \text{ of moderate stained cells}) + (3 \times \% \text{ of strong stained cells})]$, giving an analytical range from 0 to 300.

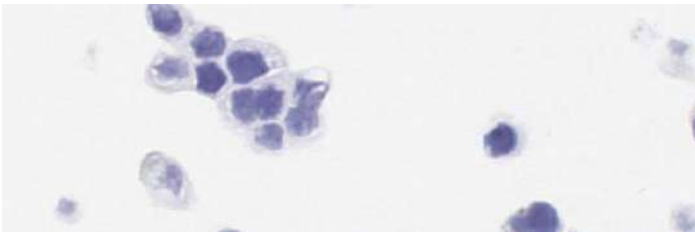
Visiopharm® is a registered trademark of Visiopharm A/S.

CLDN6 expression in FFPE cell pellets (BOND™ RX)

Below are the representative images of CLDN3, CLDN4, CLDN5, CLDN6 and CLDN9 expression in FFPE 293T (human embryonic kidney epithelial) cells. CLDN6 was detected in the CLDN6 overexpressing cell line and absent in the CLDN3, CLDN4, CLDN5, and CLDN9 overexpressing cell lines.

Formalin fixed paraffin-embedded 293T cells

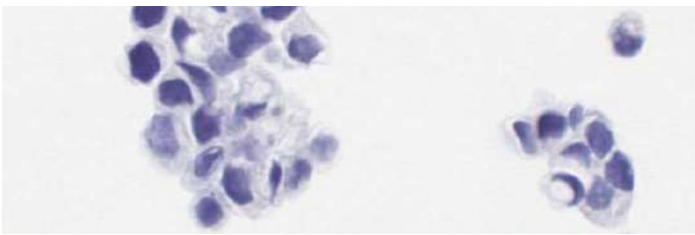
Human CLDN3 expression vector



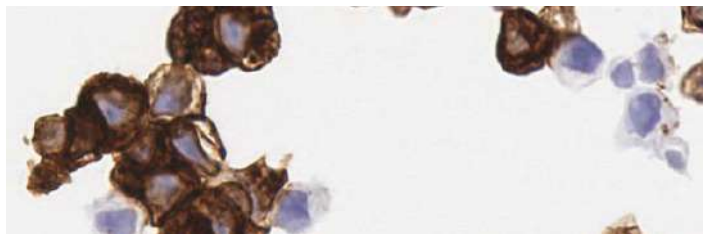
Human CLDN4 expression vector



Human CLDN5 expression vector



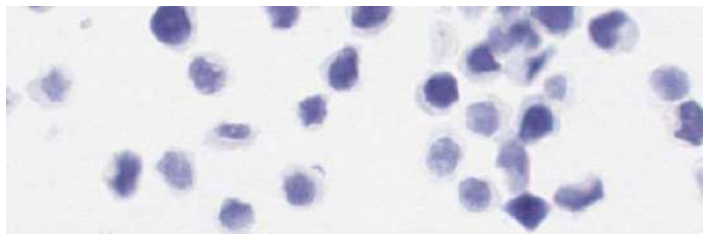
Human CLDN6 expression vector



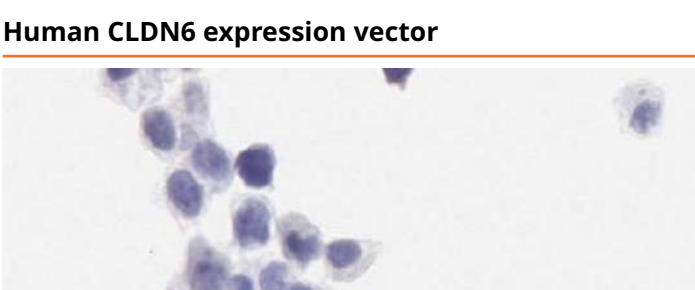
Human CLDN9 expression vector



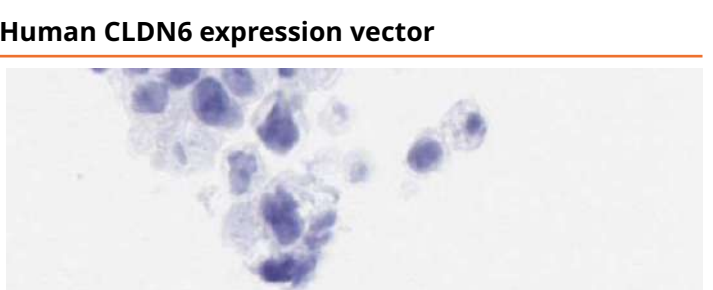
Empty expression vector



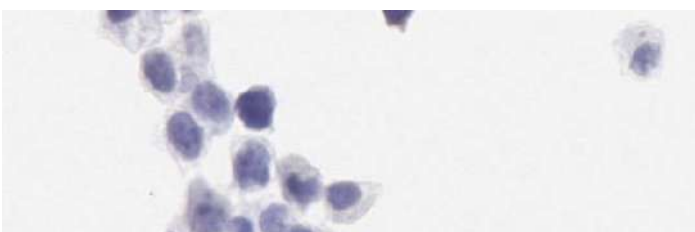
Isotype control



Secondary antibody only control



Human CLDN6 expression vector



Human CLDN6 expression vector

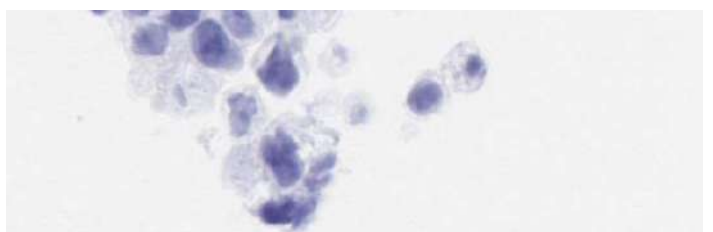


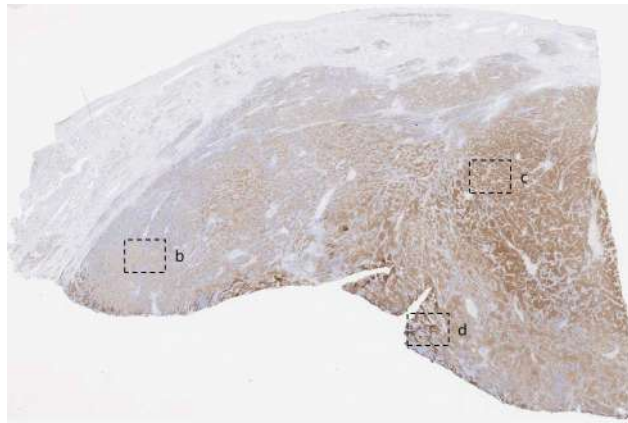
Figure 1. Immunohistochemical analysis of CLDN6 expression in FFPE 293T (human embryonic kidney epithelial cells) transfected with over expression vectors (CLDN3, CLDN4, CLDN5, CLDN6, CLDN9 and empty expression vector) containing a His tag.

IHC images show CLDN6 detection using anti- Claudin 6 recombinant antibody (ab314134) at a concentration of 0.1 µg/ml. Cells transfected with an empty vector showed no staining. Absence of staining was observed when stained using anti-rabbit IgG isotype control (ab172730). Positive staining in brown; Nuclear hematoxylin counterstain in blue. Slides were scanned at 40x on Aperio® AT2 and imaged at 40x on Aperio® ImageScope.

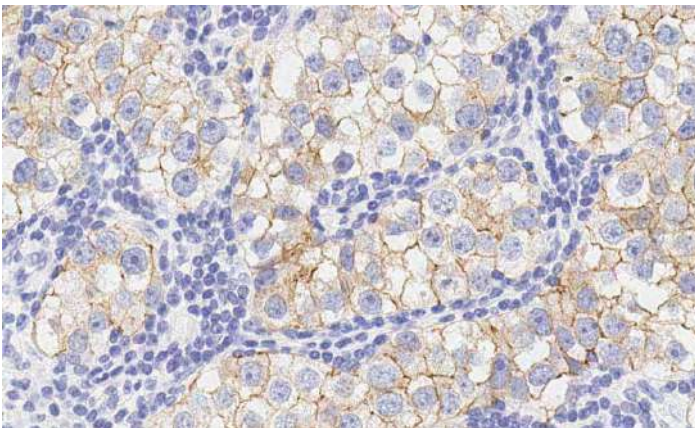
CLDN6 expression in testicular cancer (BOND™ RX)

Below are the representative images of an individual seminoma case showing heterogenous CLDN6 expression.

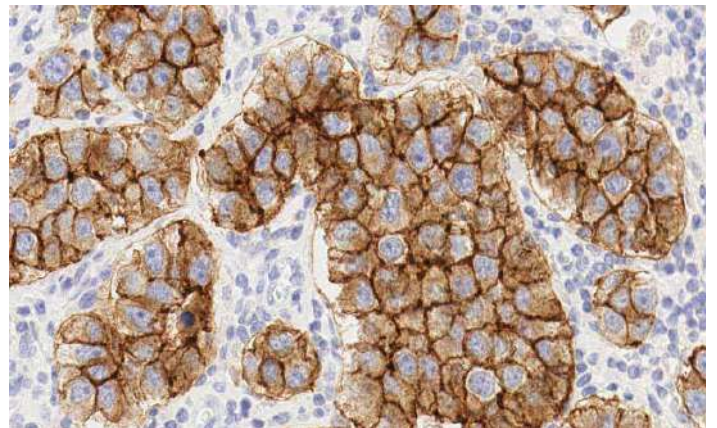
CLDN6 (a)



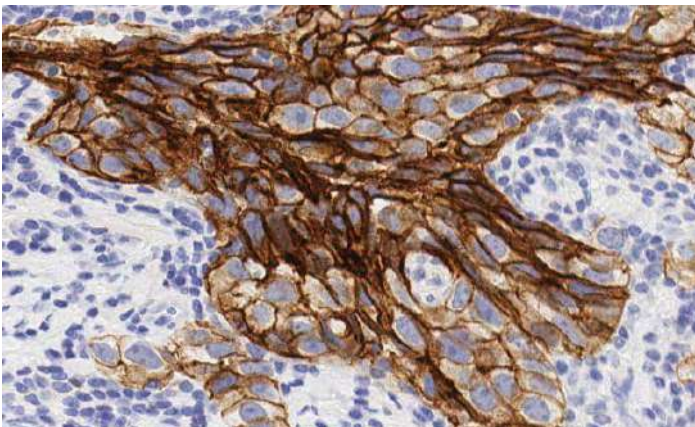
Weak expression (b)



Moderate expression (c)



Strong expression (d)



Secondary only control

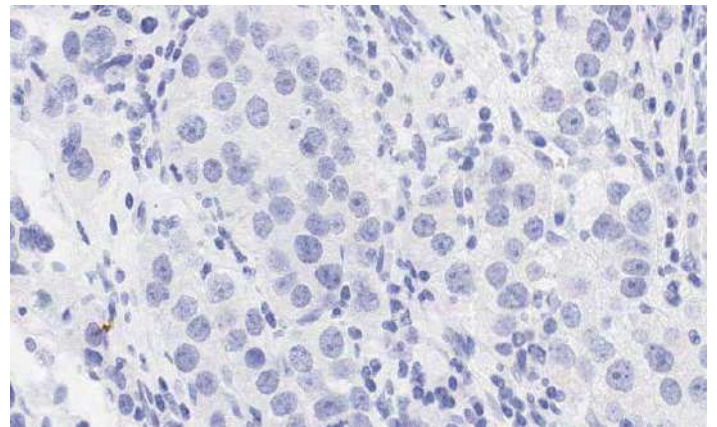


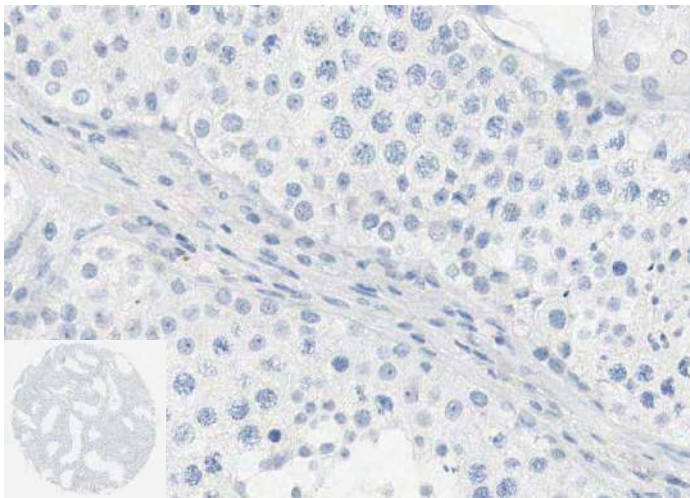
Figure 2. CLDN6 expression in testicular cancer (seminoma). IHC images show heterogenous Claudin 6 staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 0.5x (a) and 20x (b-e) on Aperio® ImageScope.

CLDN6 expression in human multi-normal TMA (DISCOVERY ULTRA)

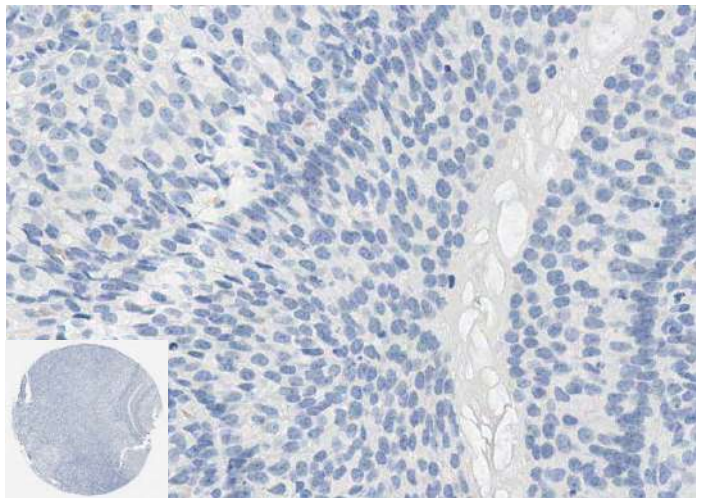
Below are the representative images of human multi-normal TMA where CLDN6 expression was not detected in any of the 34 normal human tissues.

CLDN6

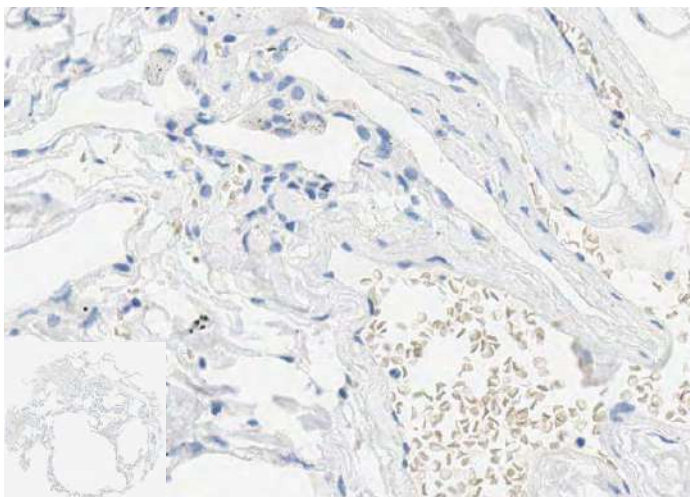
Testis



Ovary



Lung



Stomach

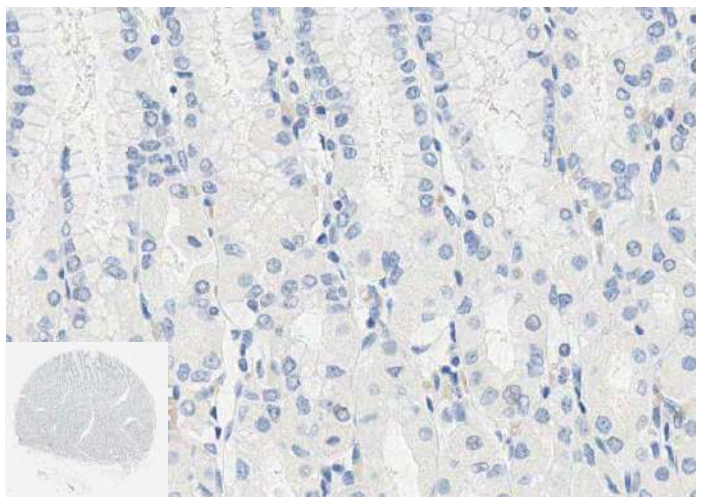


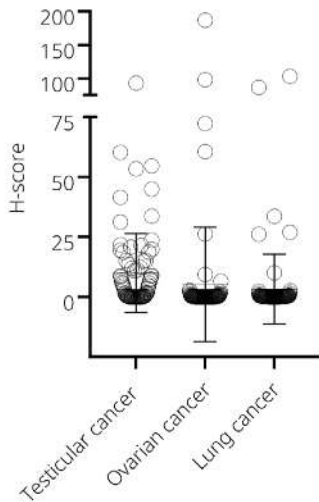
Figure 3. CLDN6 expression in human normal tissues. IHC staining of normal human testis, ovary, lung and stomach using anti-Claudin 6 (ab314134) antibody showed the absence of CLDN6 expression. Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

The multinormal TMA consisted of other tissues (not shown) such as stomach, small intestine, colon, liver, lung, spleen, umbilical cord, parathyroid, thyroid, tonsil, bone marrow, fallopian tube, kidney, pancreas, skin, thymus, endometrium, urinary bladder, breast (2x), heart, ovary, prostate, testis, cervix, adrenal gland, lymph node, skeletal muscle and ureter. As expected, CLDN6 expression was not detected.

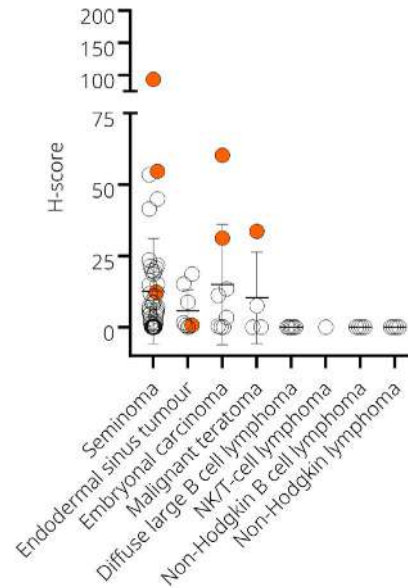
CLDN6 expression in cancer (DISCOVERY ULTRA)

CLDN6 expression varied in the analyzed cancer TMAs, with testicular cancer showing the highest H- score and lung cancer the lowest. The staining intensity of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD).

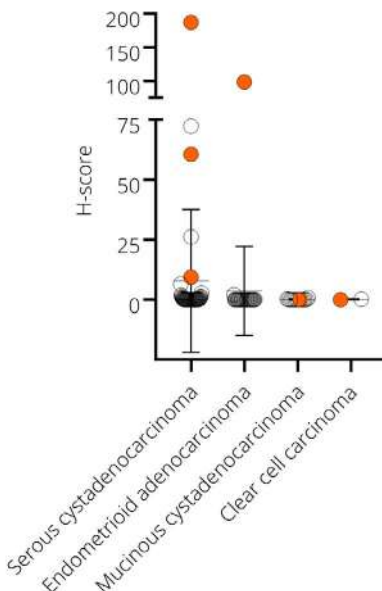
a) CLDN6 expression in selected cancer TMAs



b) CLDN6 expression in testicular cancer



c) CLDN6 expression in ovarian cancer



d) CLDN6 expression in Lung cancer

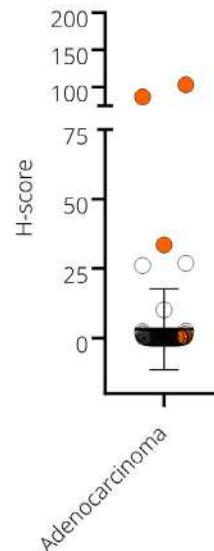


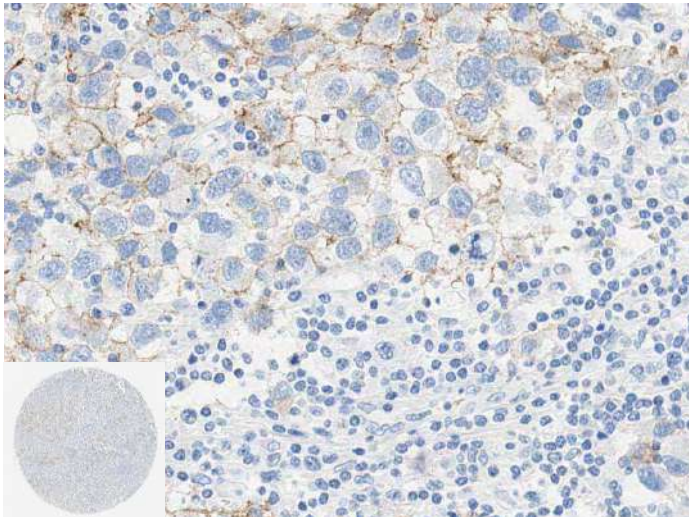
Figure 4. CLDN6 protein expression in a selection of cancer TMAs.

(a) The box plot (with SD) summarizes the mean H-score (maximum value 300) of CLDN6 expression in TMA cores. **(b)** CLDN6 H-score from 80 TMA cores of testicular cancer (Seminoma (46), Endodermal sinus tumour (8), Embryonal carcinoma (8), malignant teratoma (4), diffuse large B cell lymphoma (6), NK/T-cell lymphoma (1), non-Hodgkin lymphoma (4) and non-Hodgkin B cell lymphoma (3)). **(c)** CLDN6 H-score from 92 TMA cores of ovarian cancer (Serous cystadenocarcinoma (48), Mucinous cystadenocarcinoma (14), Endometrioid adenocarcinoma (28), Clear cell carcinoma (2)). **(d)** CLDN6 H-score from 94 cores of lung cancer (adenocarcinoma (94)). The IHC images corresponding to orange data points are shown in Figures 6-8.

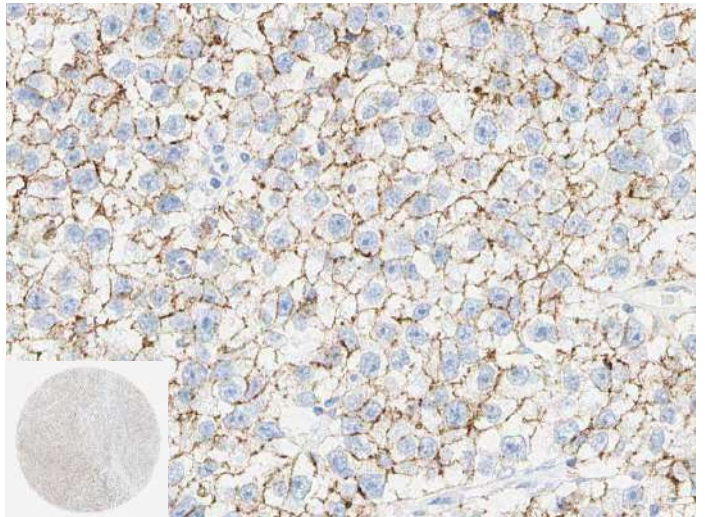
CLDN6 expression in testicular cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human testicular cancer TMA showing weak to strong CLDN6 expression.

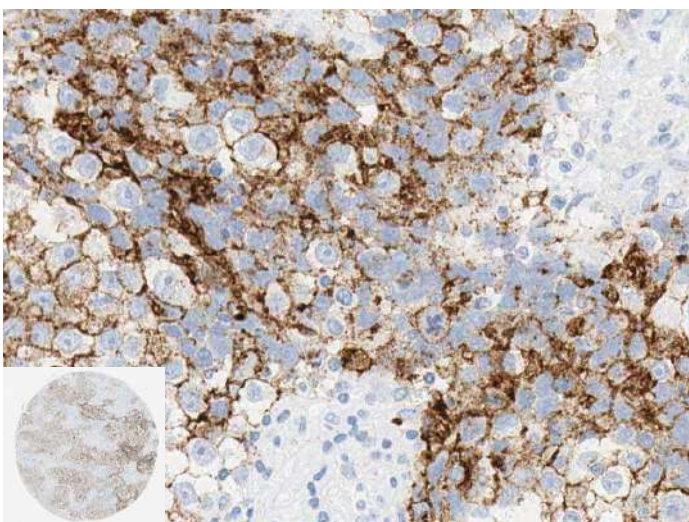
Seminoma
(H-score 12.29)



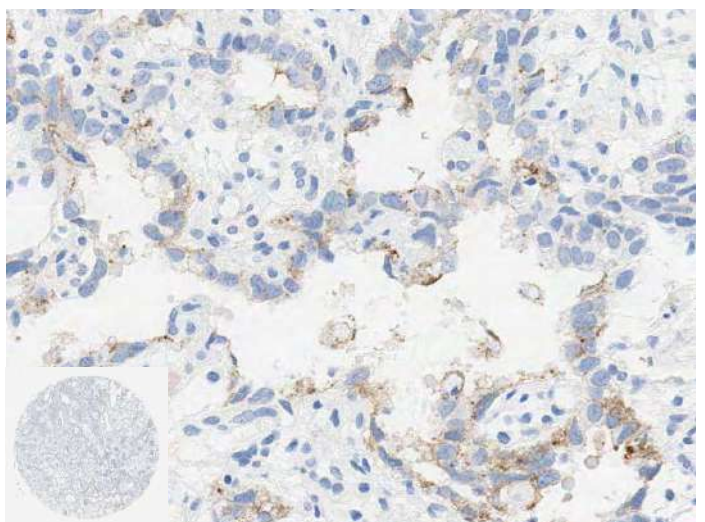
Seminoma
(H-score 54.63)



Seminoma
(H-score 93.22)



Endodermal sinus tumour
(H-score 0.72)



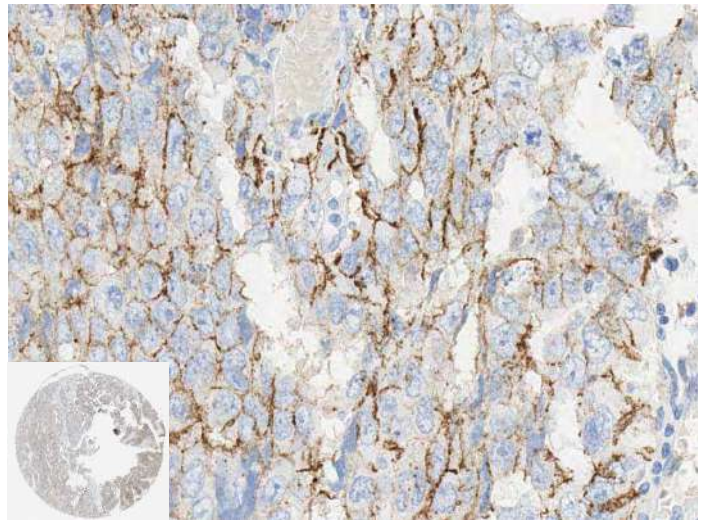
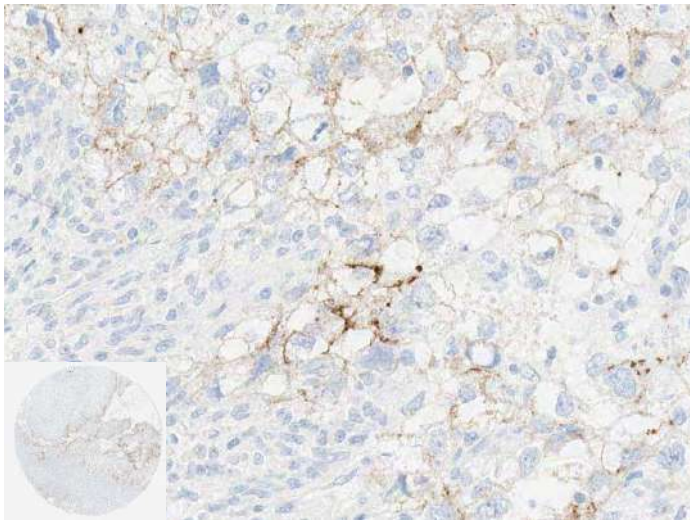
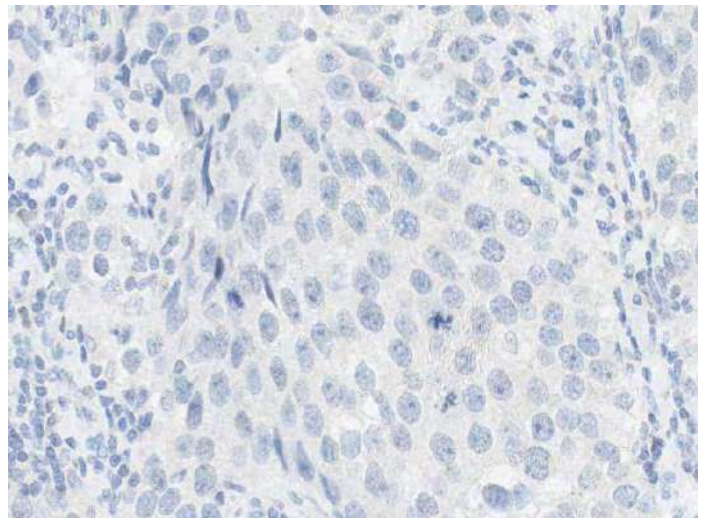
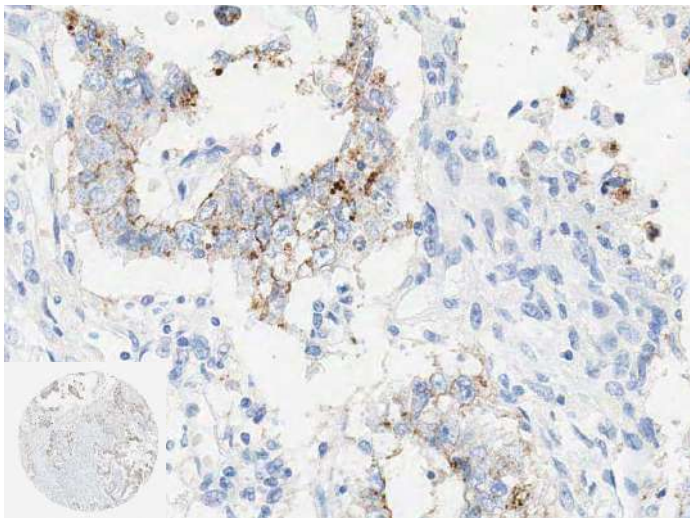
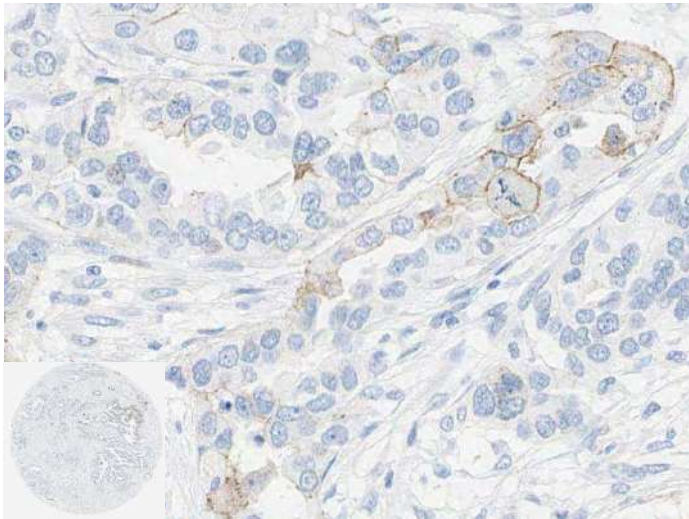
Embryonal carcinoma
(H-score 31.19)**Embryonal carcinoma**
(H-score 60.29)**Malignant teratoma**
(H-score 33.63)**Seminoma**
(Isotype control)

Figure 5. CLDN6 expression in testicular cancer. IHC images show weak, moderate or strong Claudin 6 staining in brown (a-g); absence of staining was observed when stained using anti-rabbit IgG isotype control (ab172730) (h). 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.

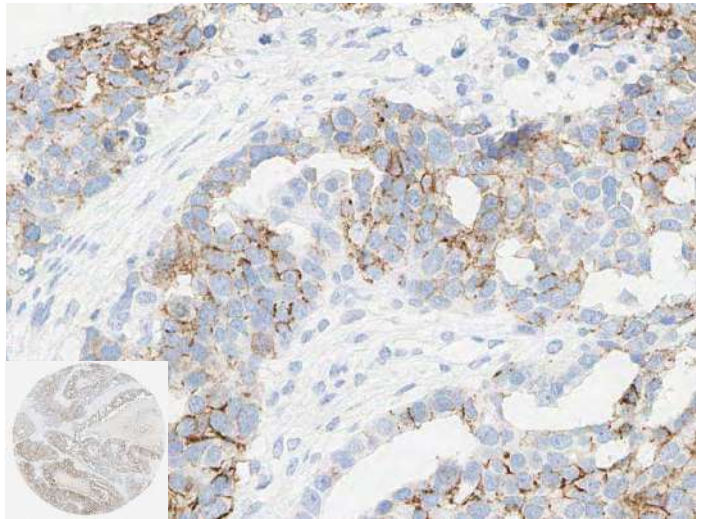
CLDN6 expression in ovarian cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human ovarian cancer TMA showing weak to strong CLDN6 expression.

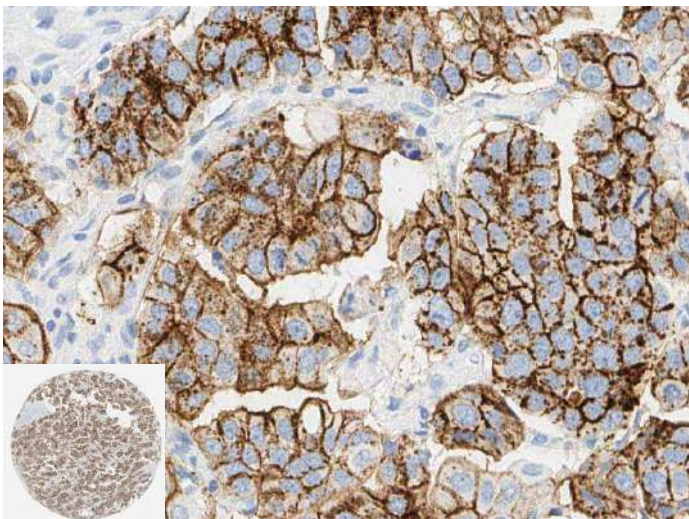
Serous cystadenocarcinoma
(H-score 9.38)



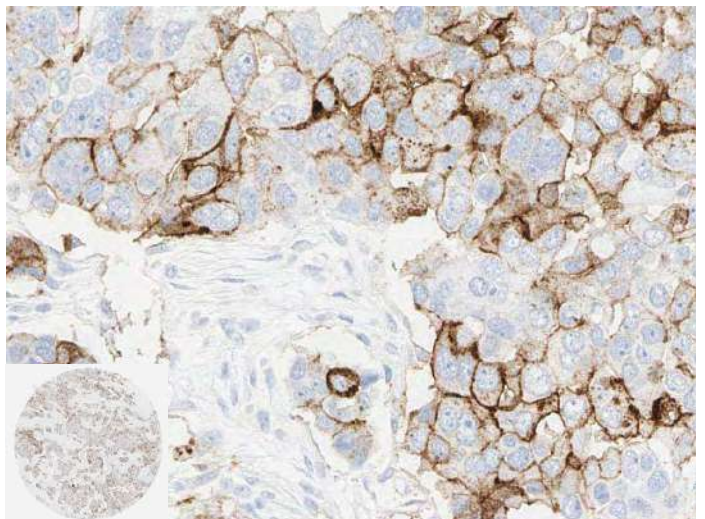
Serous cystadenocarcinoma
(H-score 60.57)



Serous cystadenocarcinoma
(H-score 186.92)



Endometrioid adenocarcinoma
(H-score 98.28)



Mucinous cystadenocarcinoma
(H-score 0.06)

Clear cell carcinoma
(H-score 0.02)

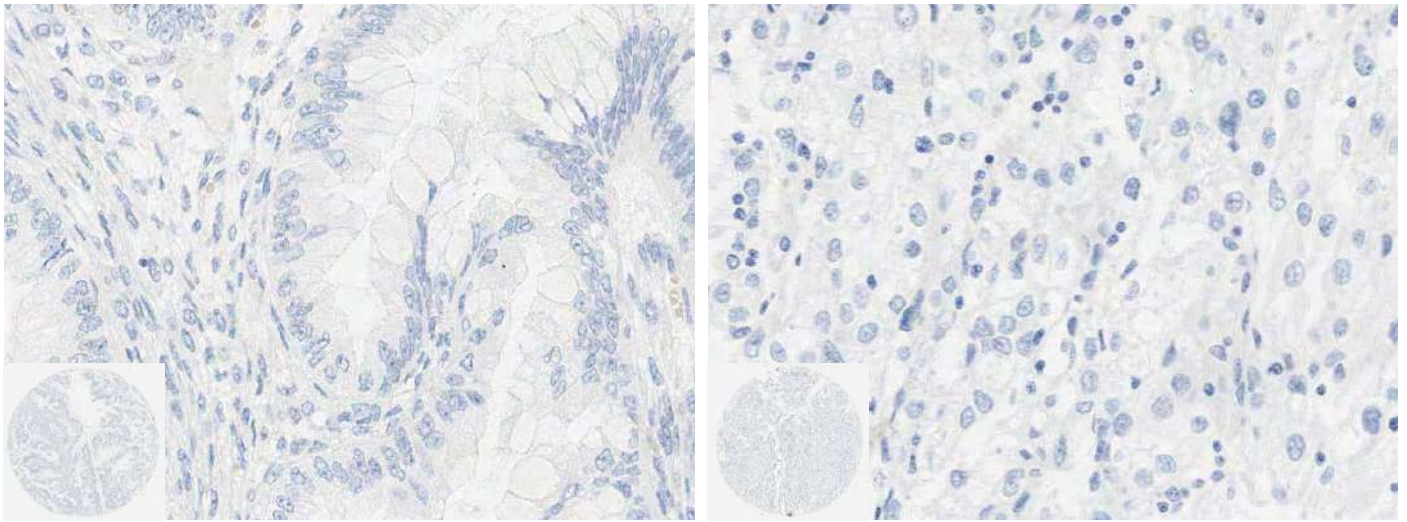
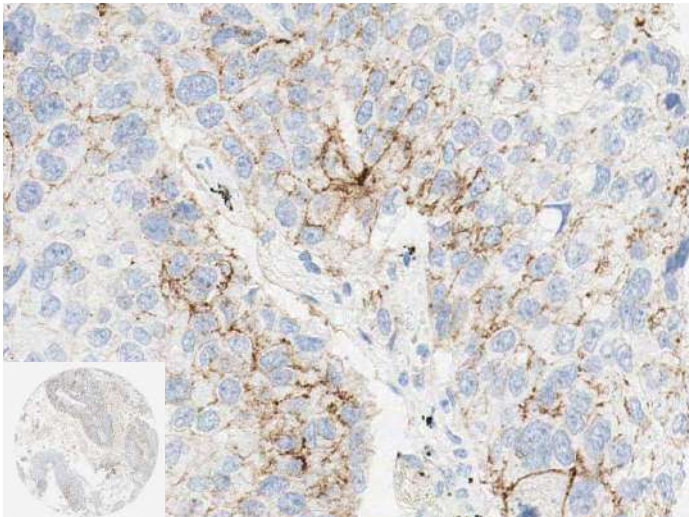


Figure 6. CLDN6 expression in ovarian cancer. IHC images show weak, moderate or strong Claudin 6 staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

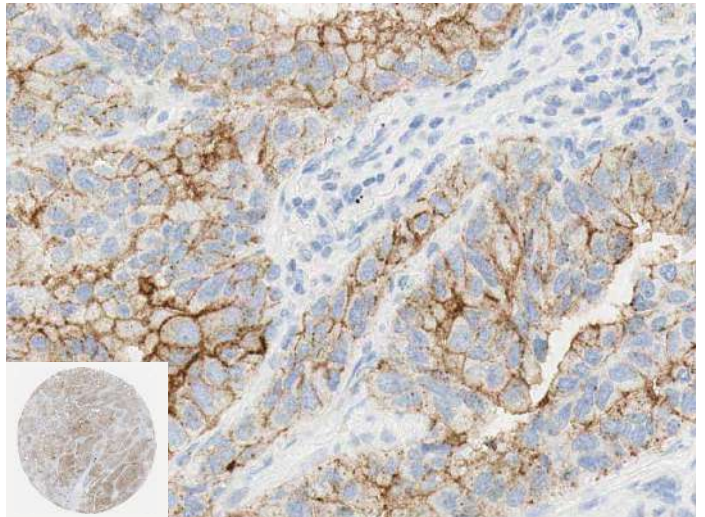
CLDN6 expression in lung cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human lung cancer TMA showing weak to strong CLDN6 expression.

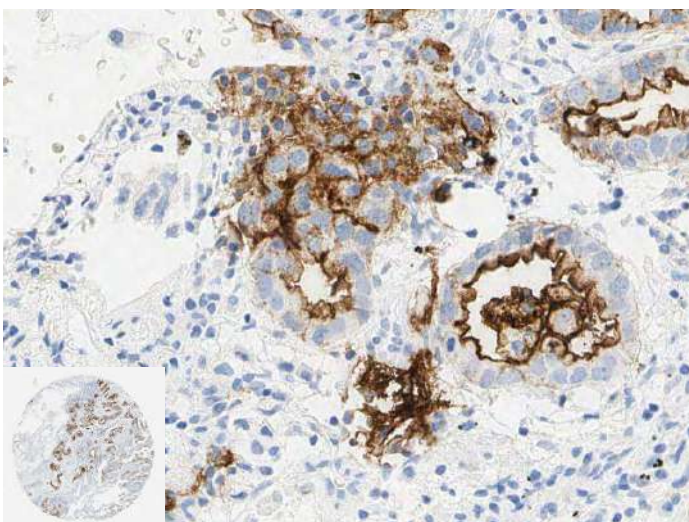
Adenocarcinoma
(H-score 33.51)



Adenocarcinoma
(H-score 86.48)



Adenocarcinoma
(H-score 102.99)



Adenocarcinoma
(H-score 0.74)

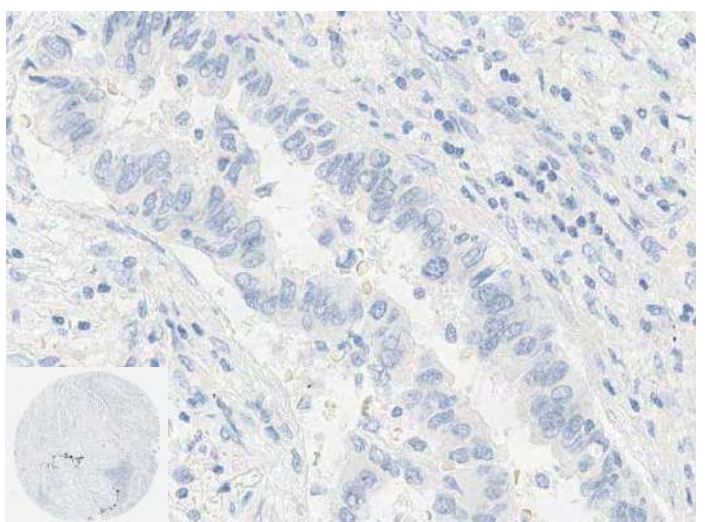


Figure 7. CLDN6 expression in lung cancer. IHC images show weak, moderate or strong Claudin 6 staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio™ ImageScope.

References

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3. Chiba, Hideki et al. "Hepatocyte nuclear factor (HNF)-4alpha triggers formation of functional tight junctions and establishment of polarized epithelial morphology in F9 embryonal carcinoma cells." *Experimental cell research* vol. 286,2 (2003): 288-97. doi:10.1016/s0014-4827(03)00116-2
4. Anderson, William J et al. "Genetic targeting of the endoderm with claudin-6CreER." *Developmental dynamics: an official publication of the American Association of Anatomists* vol. 237,2 (2008): 504-12. doi:10.1002/dvdy.21437
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6. Sullivan, Lisa M et al. "Claudin-6 is a nonspecific marker for malignant rhabdoid and other pediatric tumors." *The American journal of surgical pathology* vol. 36,1 (2012): 73-80. doi:10.1097/PAS.0b013e31822cfa7e
7. Ushiku, Tetsuo et al. "Distinct expression pattern of claudin-6, a primitive phenotypic tight junction molecule, in germ cell tumours and visceral carcinomas." *Histopathology* vol. 61,6 (2012): 1043-56. doi:10.1111/j.1365-2559.2012.04314.x
8. Kojima, Manabu et al. "Prognostic Significance of Aberrant Claudin-6 Expression in Endometrial Cancer." *Cancers* vol. 12,10 2748. 24 Sep. 2020, doi:10.3390/cancers12102748

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oncology@abcam.com

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abcam

