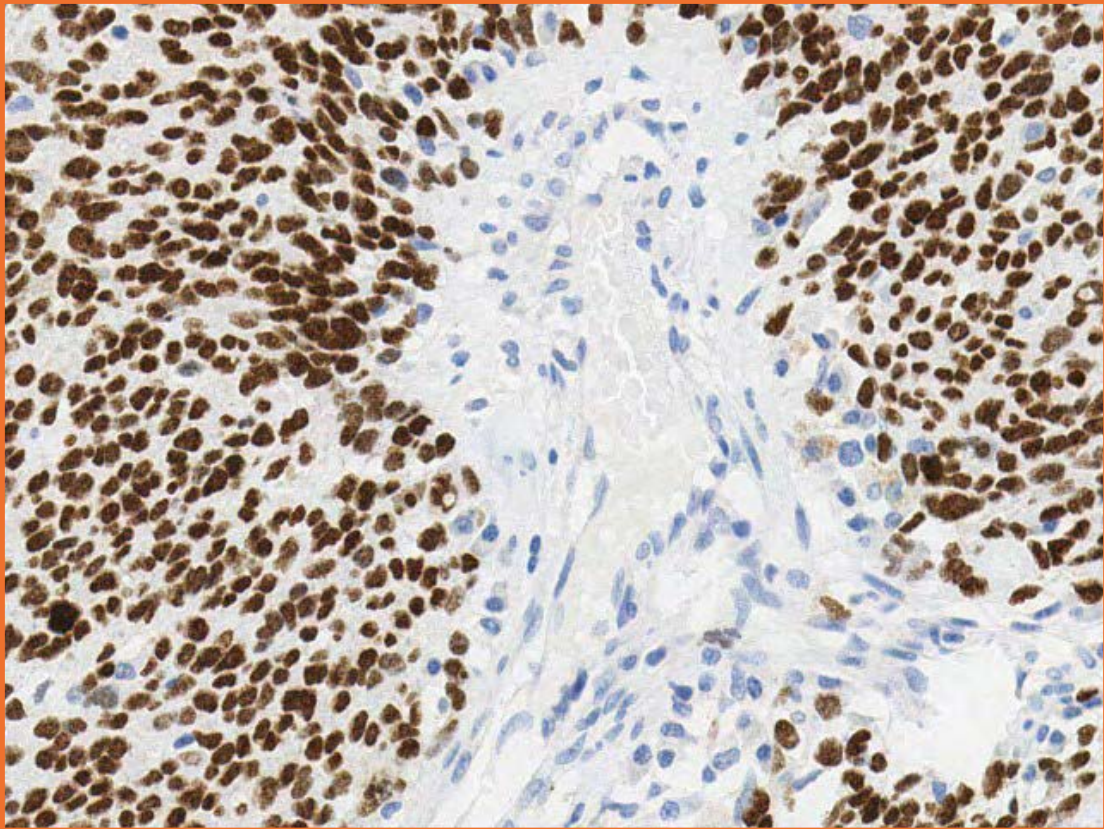


**Enhanced validation data**

**Anti-SOX10 recombinant  
antibody – ab180862**



# Enhanced validation of Anti-SOX10 recombinant antibody [EPR4007-104] – ab180862

## 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-SOX10 recombinant antibody (ab180862) in IHC in TMAs using a BOND™ RX Research Stainer (Leica®) and DISCOVERY ULTRA system (Roche Diagnostics).
- A quantitative H-score analysis of SOX10 expression was performed using the artificial intelligence (AI)-driven digital image analysis software Visiopharm® (Visiopharm A/S).

## Target overview

### HGNC symbol

SOX10

### Approved name

SRY-box transcription factor 10

### Chromosomal location

22q13.1

## Function

- Functions as a transcription factor that plays a multifaceted role from embryonic development to pathogenesis<sup>(1,2)</sup>.
- Mutations in SOX10 disrupt neural crest development, resulting in a variety of disorders such as PCWH syndrome (Peripheral demyelinating neuropathy, central dysmyelination, Waardenburg syndrome, and Hirschsprung disease)<sup>(3-12)</sup>.
- In neural and neuroectodermal tumors, SOX10 plays a key role in proliferation and differentiation, making it a valuable diagnostic and therapeutic molecule<sup>(13-16)</sup>.

## Tissue specificity

- Expression is elevated in normal tissues such as the brain, skin, breast, salivary gland and in cancer such as melanoma, gliomas and breast cancer.

## Cellular localization

- Localized to the nucleus.

Target information above in part from: UniProt accession [P56693](#)

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. mRNA expression was further analyzed for oncology targets in cBioPortal for Cancer Genomics using the Cancer Genome Atlas (TCGA) PanCancer Atlas datasets<sup>(17-20)</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
Nervous system gliomas	96	48	3	45	Pantomics (#NGL961)
Melanoma	102	102	22	80	Pantomics (#MEL1021)
Breast cancer	96	96	0	96	Pantomics (#BRC964)

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

a) The multinormal 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. The placenta and liver were from a single donor.

b) The multicancer TMA consists of the following tissues from two donors: seminoma, prostate adenocarcinoma, bladder carcinoma, renal cell carcinoma, melanoma, stomach adenocarcinoma, pancreatic adenocarcinoma, hepatocellular carcinoma, ovarian 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-SOX10 antibody [EPR4007-104]-ab180862 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 1.0 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).

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

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-SOX10 antibody [EPR4007-104]-ab180862 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 0.25 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

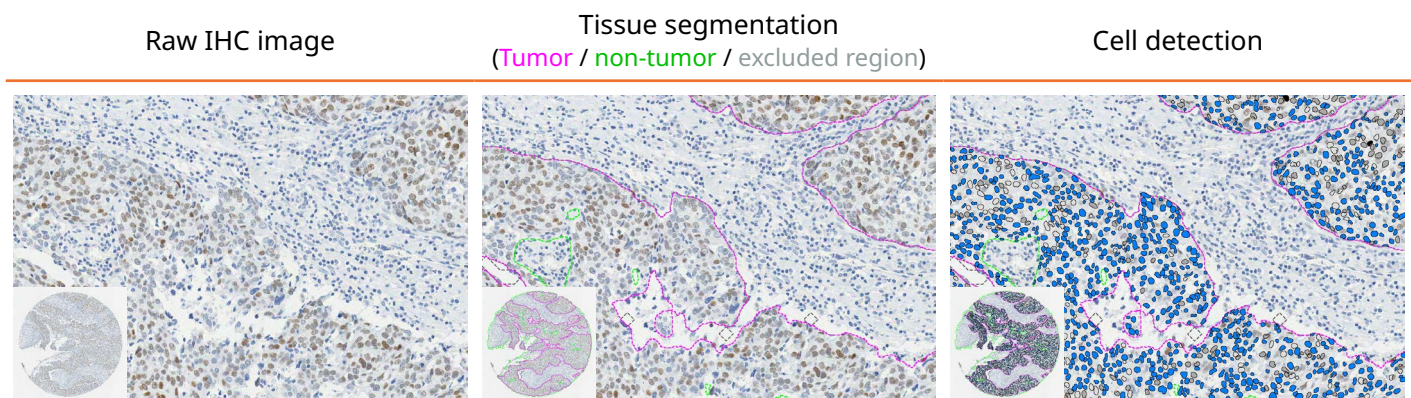
**Table 3 . IHC staining protocol on BOND™ RX Research Stainer (Leica®).** The protocol used is the same as the default IHC protocol F on BOND™ RX Research Stainer (Leica®), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature unless otherwise stated.

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 SOX10 expression was performed using the artificial intelligence (AI)-driven digital image analysis software Visiopharm® (Version: 2023.09). TMA slides were de-arrayed and segmented (melanoma and breast cancer only) into tumor and non-tumor using a trained AI model with DeepLabv3+ architecture. The segmentation performance was assessed by eye and adjusted where necessary. Exclusion criteria included missing tissue cores (all TMAs) and the impact of high melanin content on cell detection (Melanoma TMA).

Total cell numbers for each core were counted using a trained AI model with U-Net architecture. Using the cell analysis data and thresholds, the H-score of the tumor compartment for the melanoma (#MEL1021) and breast cancer (#BRC964) TMAs and total core content for the glioblastoma (#NGL961) TMA were calculated in Visiopharm®, and the graphical representation was generated using the GraphPad Prism 10.



Raw IHC images were segmented into tumor and non-tumor regions, followed by total cell detection (tumor compartment) to determine staining intensity. There are four intensity scores shown here: blue (0), white (1+), grey (2+) and black (3+).

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

**Table 4. 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.

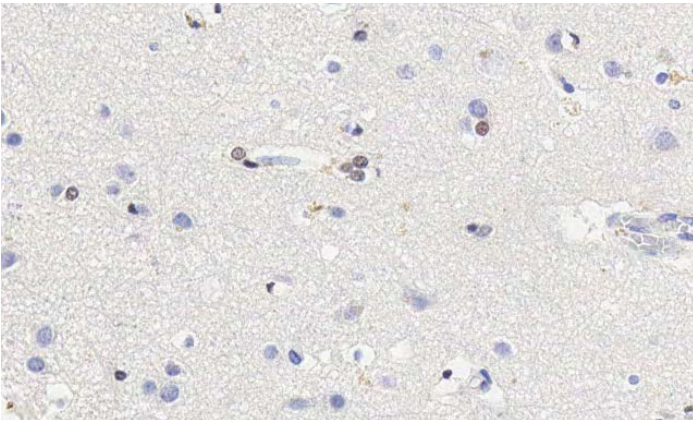
Visiopharm® is a registered trademark of Visiopharm A/S.

## SOX10 expression in multi-normal TMA (BOND™ RX)

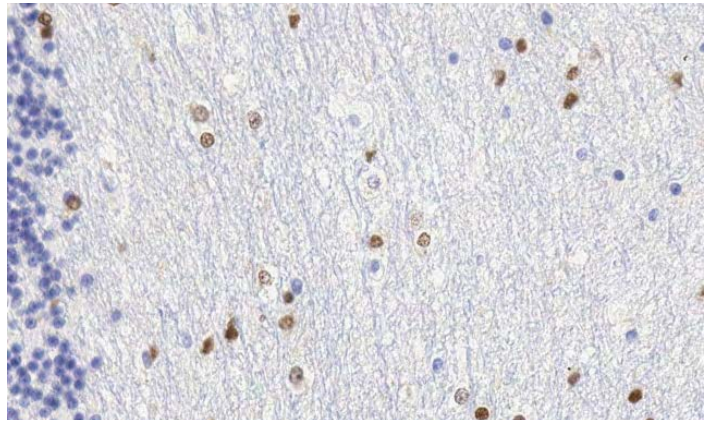
Below are the representative images of selected tissues from multi-normal TMA. SOX10 expression was detected in brain (cerebrum and cerebellum), breast and skin. Expression was absent from the heart, tonsil, kidney, stomach, small intestine, spleen and testis.

### SOX10

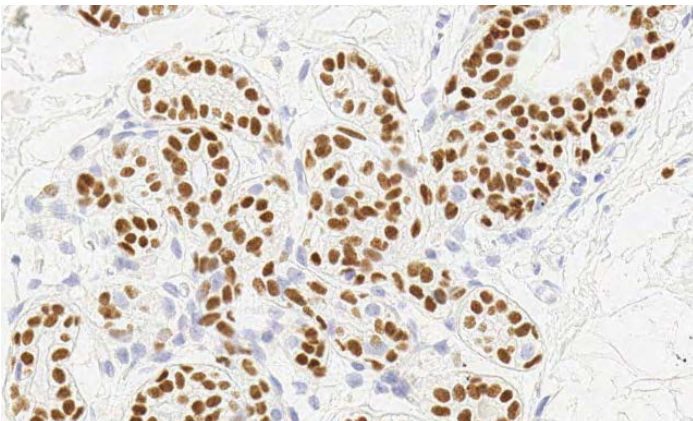
Brain (Cerebrum)



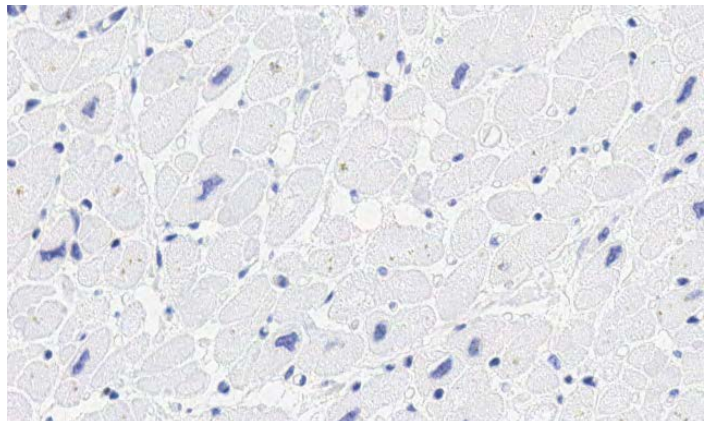
Brain (Cerebellum)



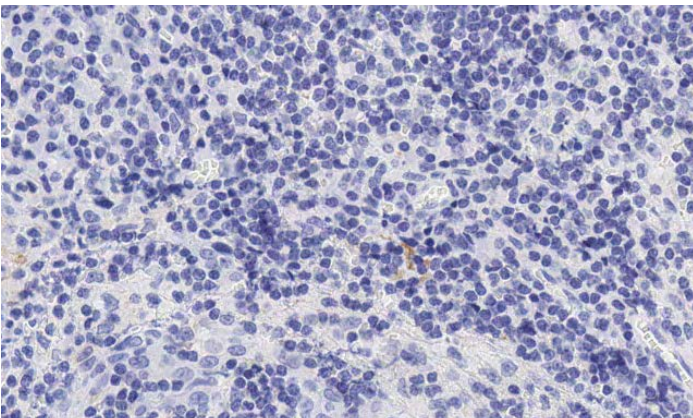
Breast



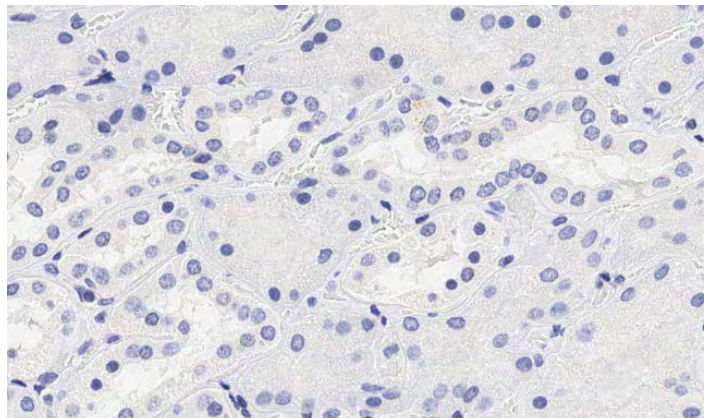
Heart

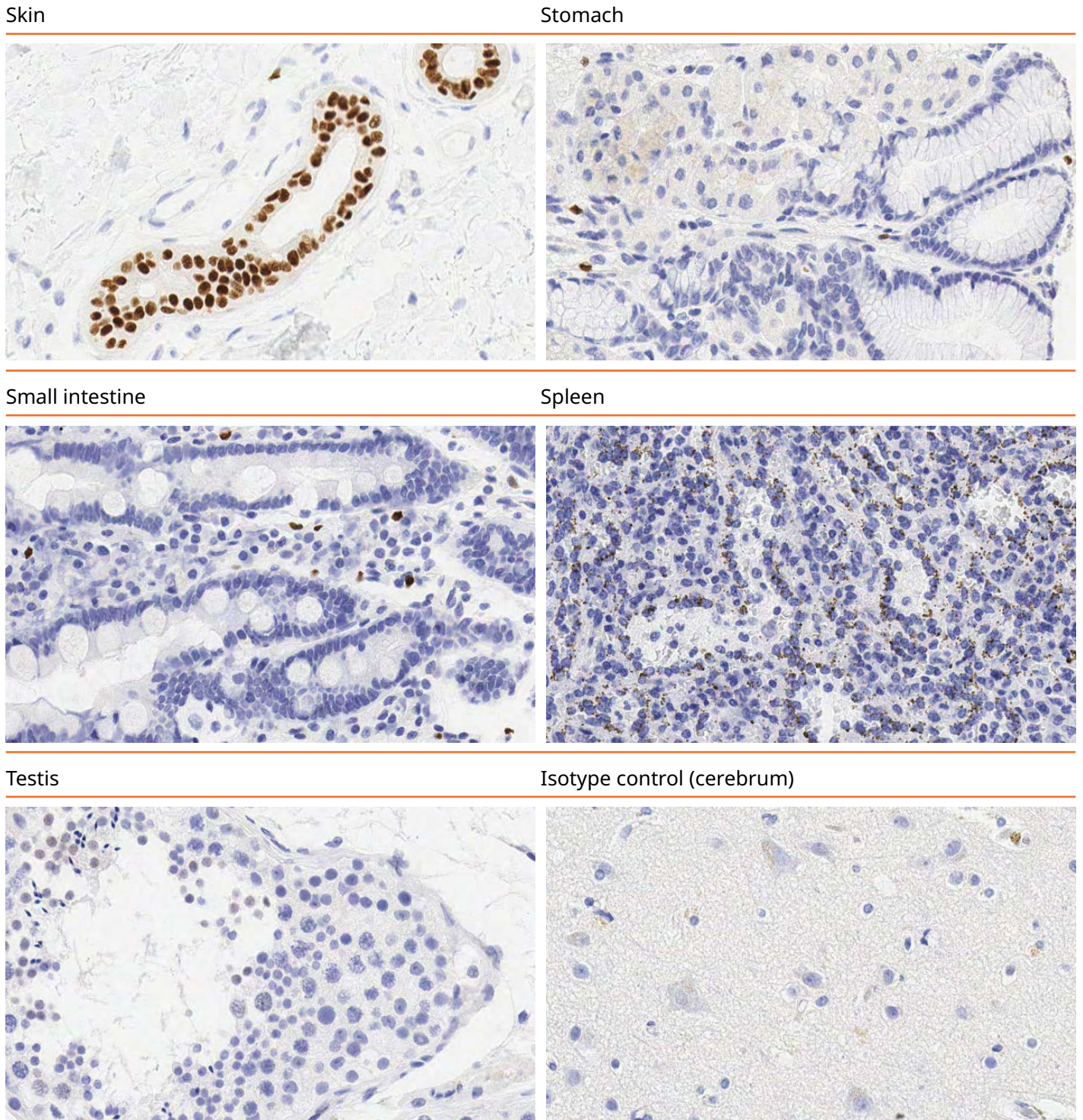


Tonsil



Kidney



**SOX10**

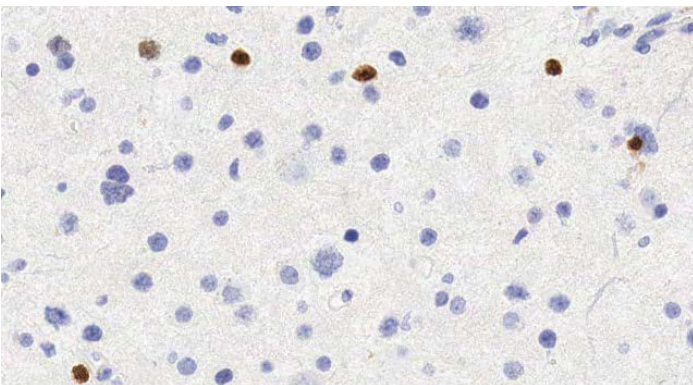
**Figure 1. SOX10 expression in human normal tissue.** IHC staining of multi-normal human tissues using anti-SOX10 (ab180682) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## SOX10 expression in multi-cancer TMA (BOND™ RX)

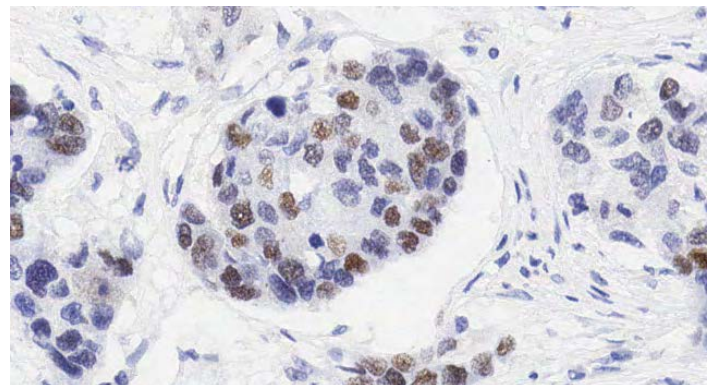
Below are the representative images of selected tissues from multi-cancer TMA. SOX10 expression was detected in low grade gliomas, breast fibroadenoma and melanoma. Expression was absent from the tissues like seminoma, stomach adenocarcinoma, T cell lymphoma, pancreatic cancer, pancreatic cancer, lung squamous cell carcinoma, endometrial cancer, bladder carcinoma, and ovarian carcinoma.

### SOX10

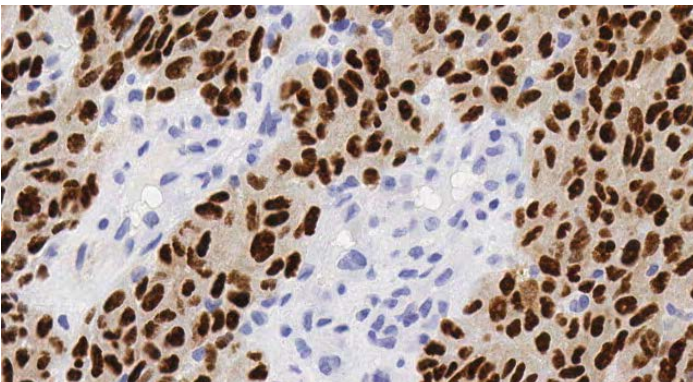
Low grade glioma



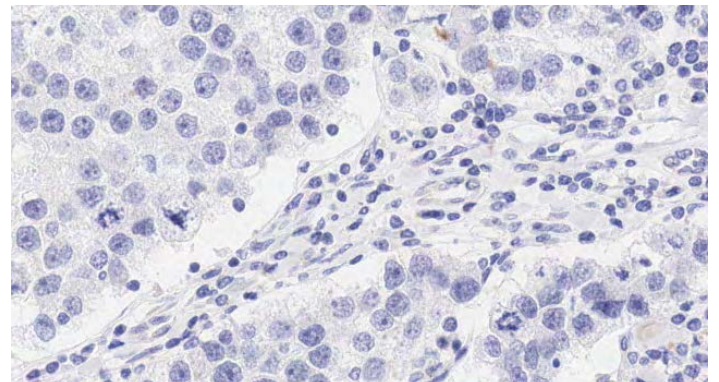
Breast fibroadenoma



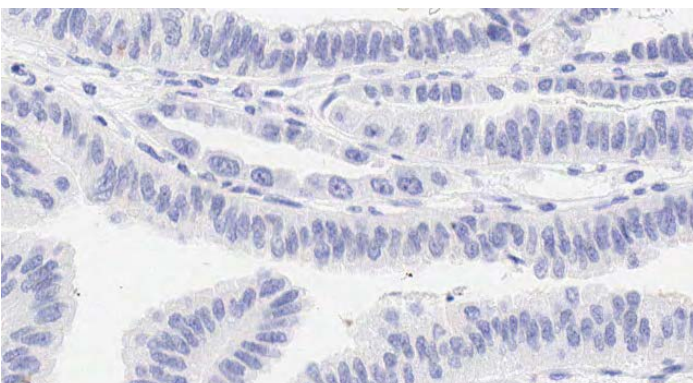
Melanoma



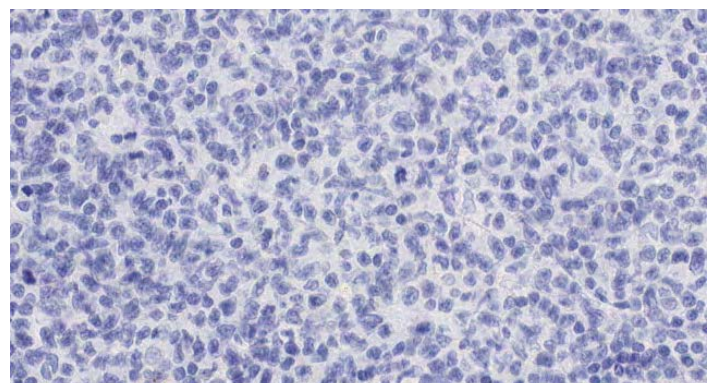
Seminoma



Stomach adenocarcinoma

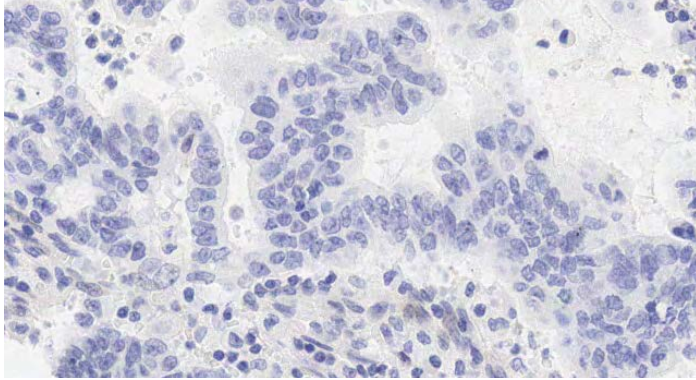


T cell lymphoma

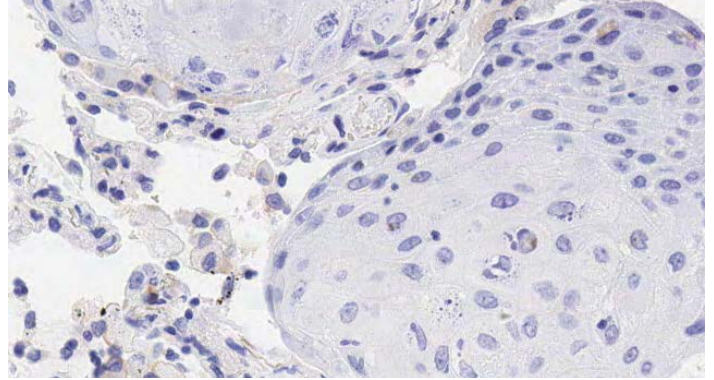


**SOX10**

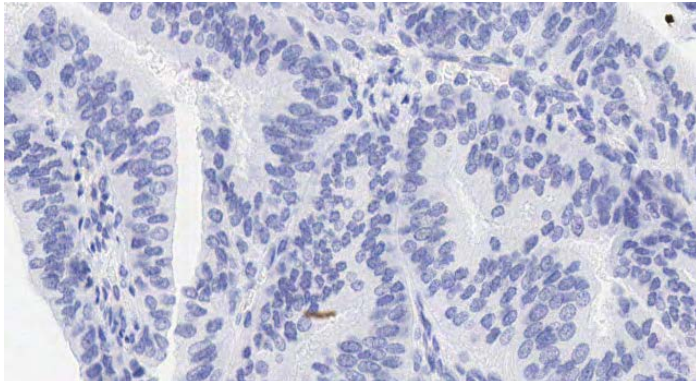
Pancreatic cancer



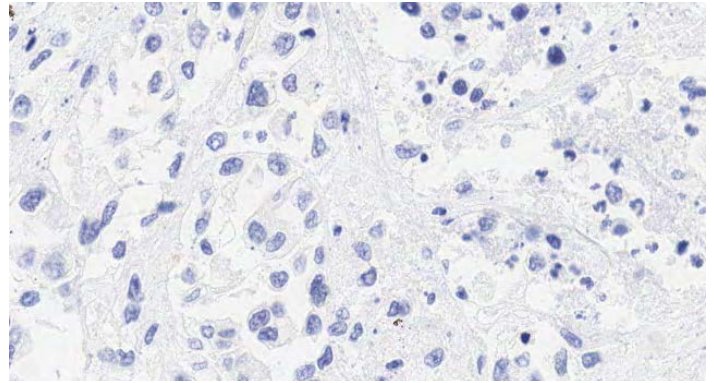
Lung squamous



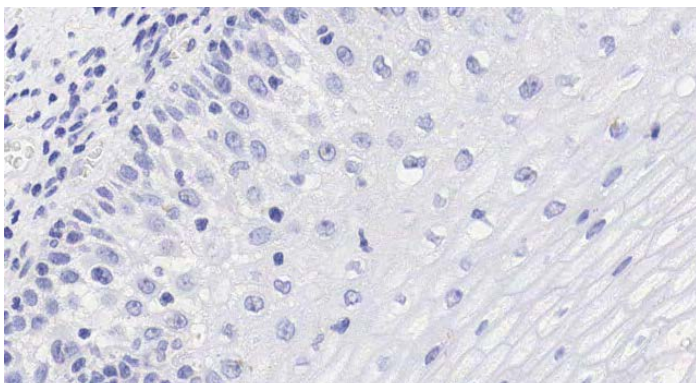
Endometrial cancer



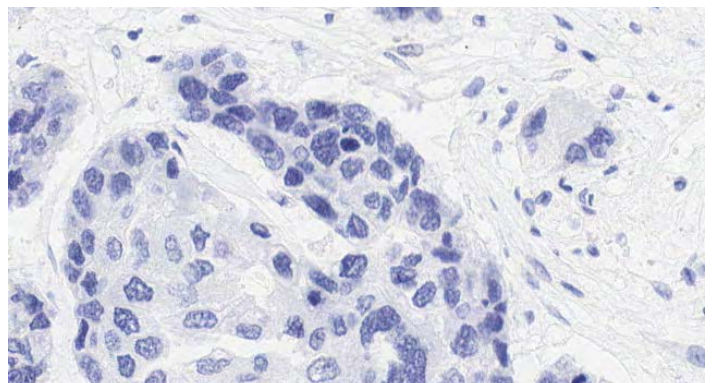
Bladder carcinoma



Ovarian carcinoma



Isotype control (Breast fibroadenoma)



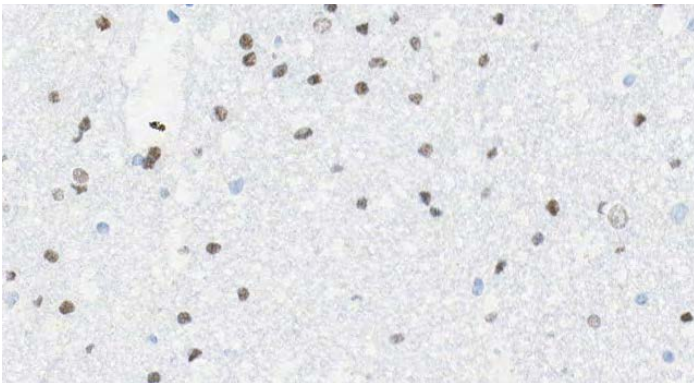
**Figure 2. SOX10 expression in cancer.** IHC staining of multi-cancer human tissues using anti-SOX10 (ab180682) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## SOX10 expression in multi-normal TMA (DISCOVERY ULTRA)

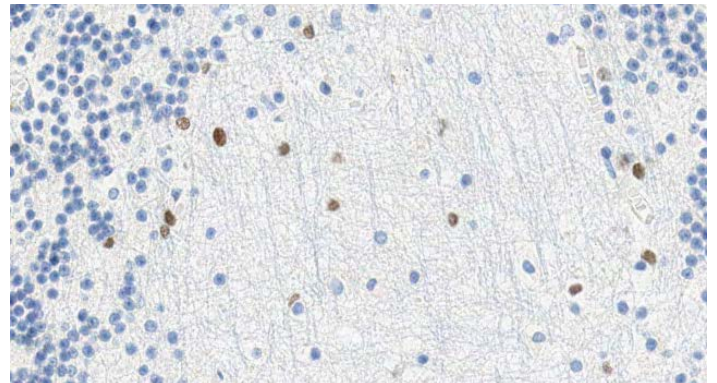
Below are the representative images of selected tissues from multi-normal TMA. SOX10 expression was detected in brain (cerebrum and cerebellum), breast, skin and small intestine. Expression was absent from the heart, tonsil, kidney, stomach, spleen and testis.

### SOX10

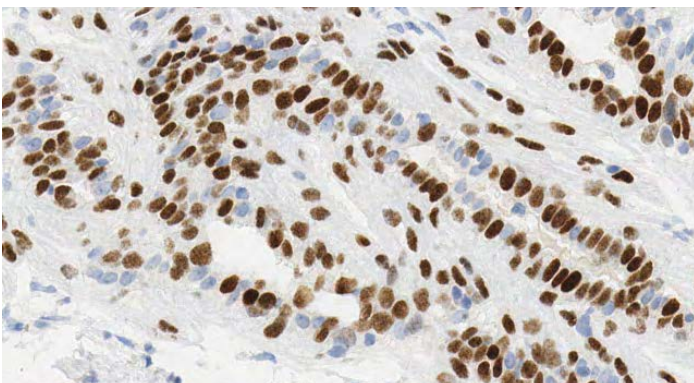
Brain (Cerebrum)



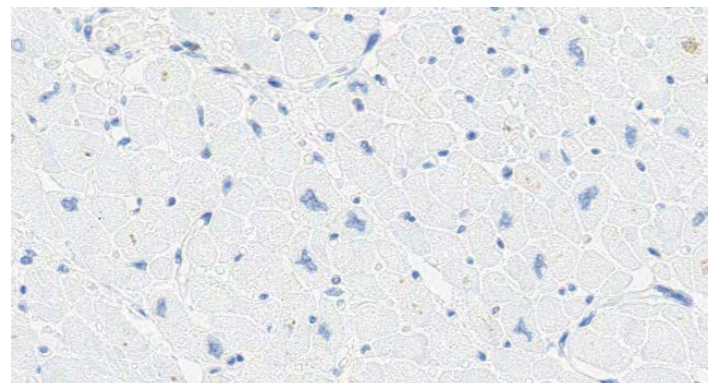
Brain (Cerebellum)



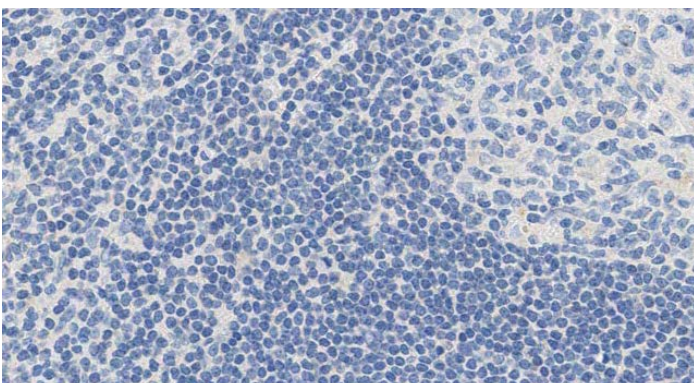
Breast



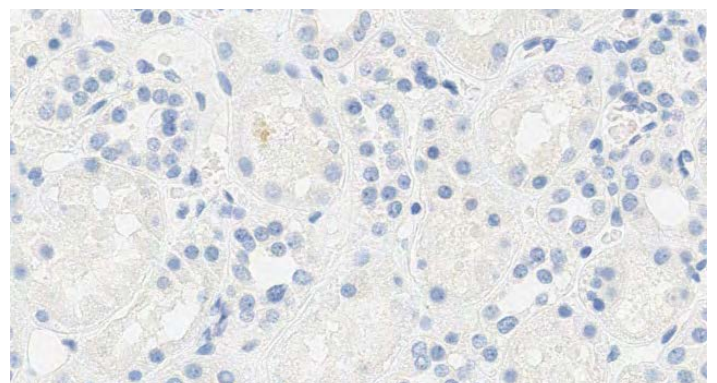
Heart

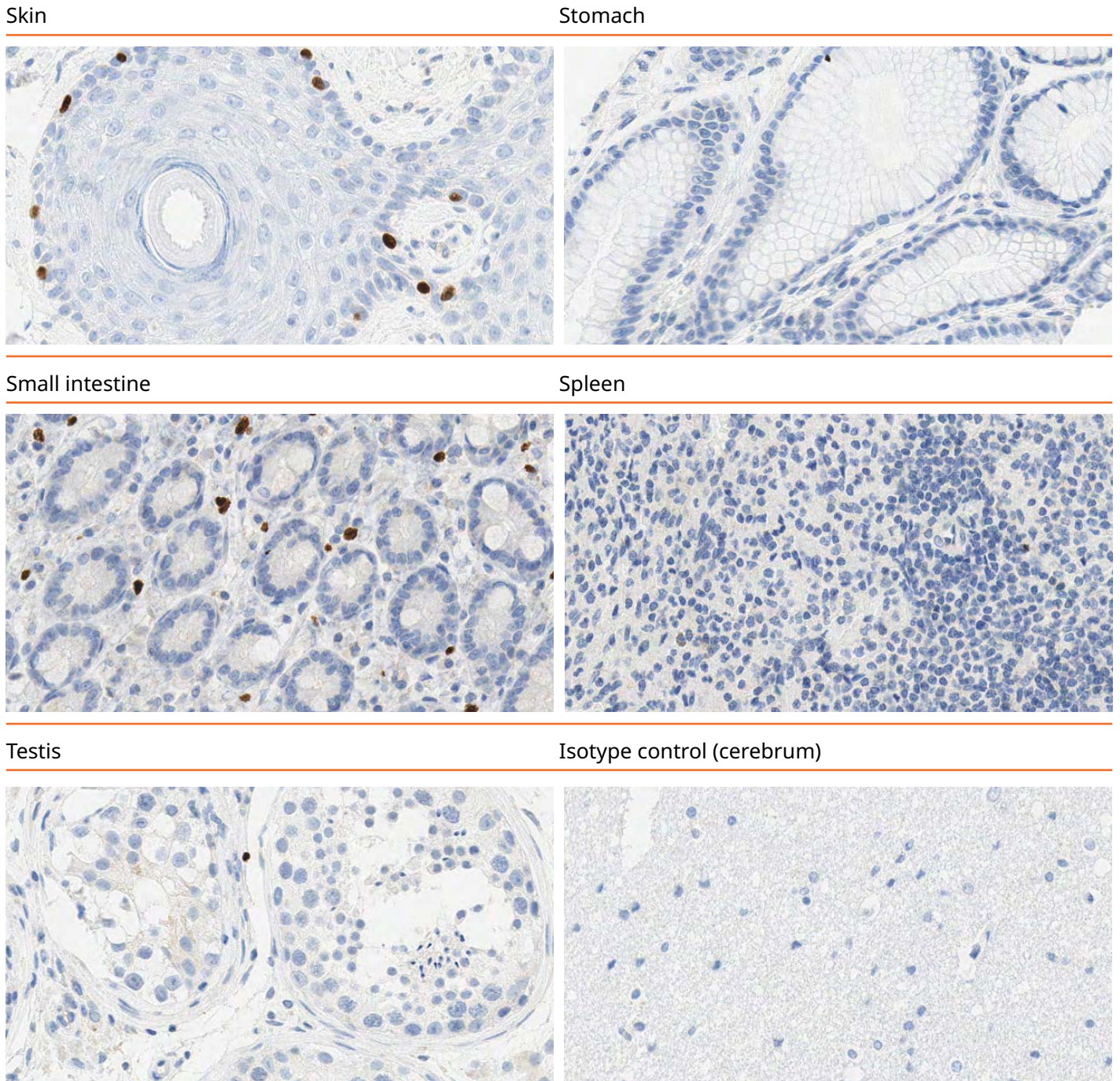


Tonsil



Kidney



**SOX10**

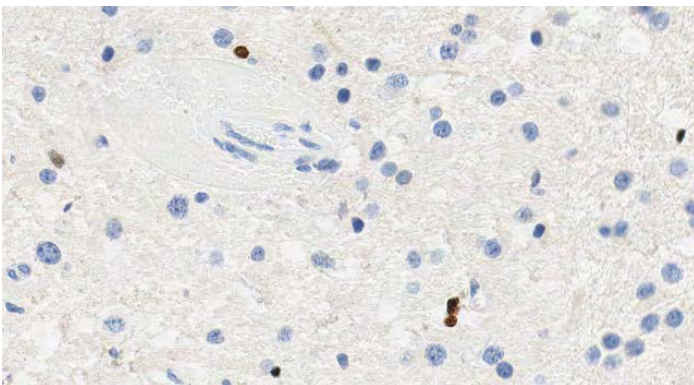
**Figure 3. SOX10 expression in human normal tissue.** IHC staining of multi-normal human tissues using anti-SOX10 (ab180682) or anti-rabbit IgG-isotype control antibody (ab172730). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

## SOX10 expression in multi-cancer TMA (DISCOVERY ULTRA)

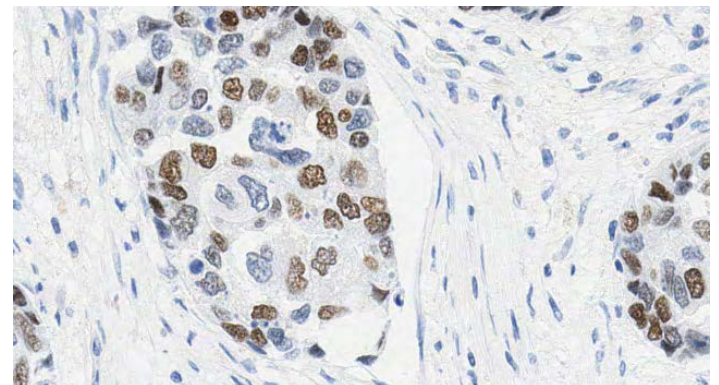
Below are the representative images of selected tissues from multi-cancer TMA. SOX10 expression was detected in low grade gliomas, breast fibroadenoma and melanoma. Expression was absent from seminoma, stomach adenocarcinoma, T cell lymphoma, pancreatic cancer, pancreatic cancer, lung squamous cell carcinoma, endometrial cancer, bladder carcinoma, ovarian carcinoma.

### SOX10

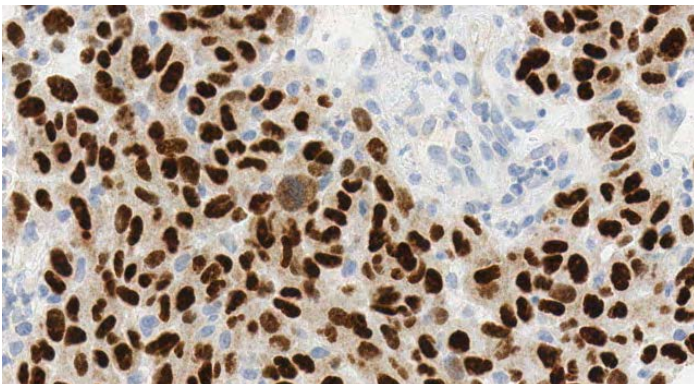
Low grade glioma



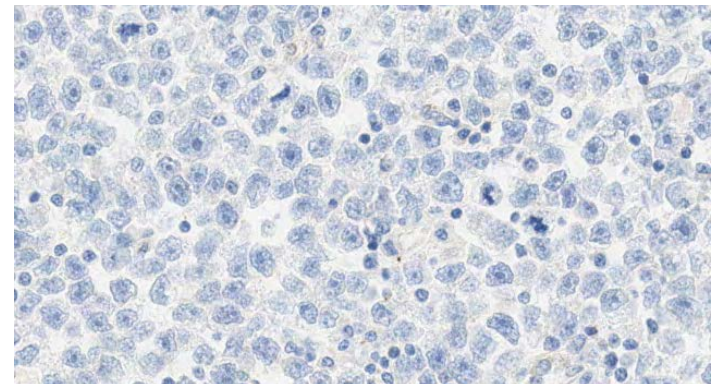
Breast fibroadenoma



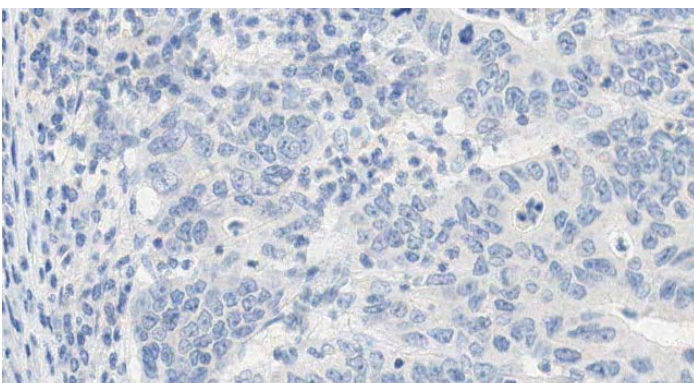
Melanoma



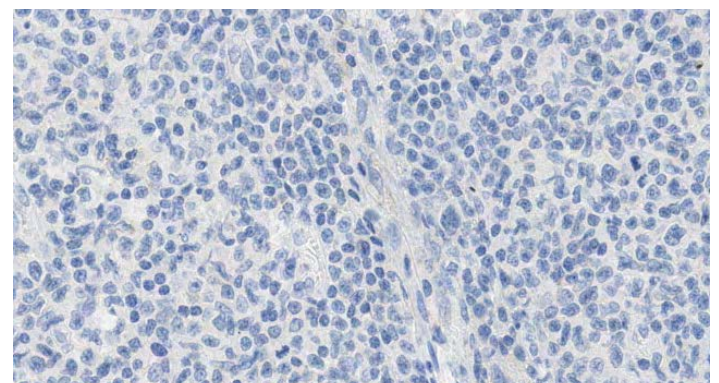
Seminoma



Stomach adenocarcinoma

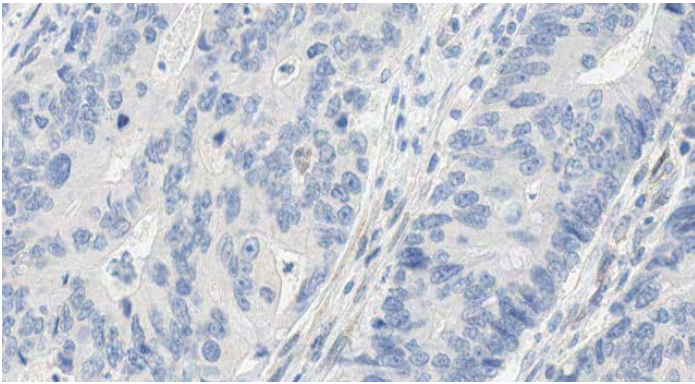


T cell lymphoma

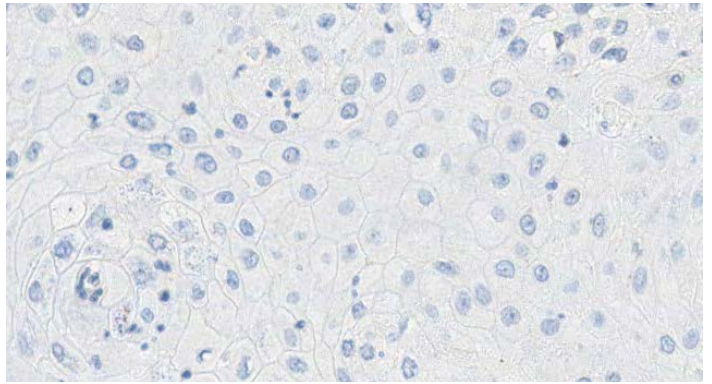


**SOX10**

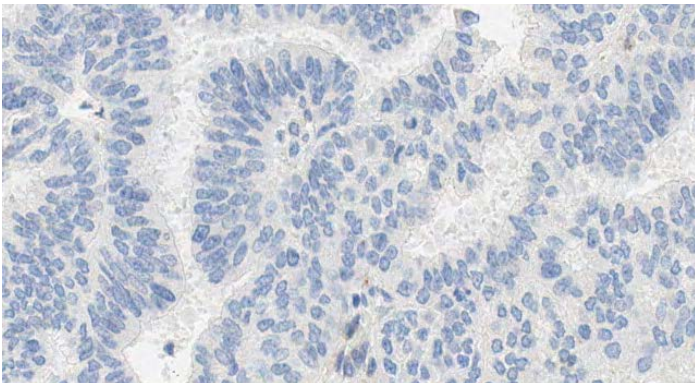
Pancreatic cancer



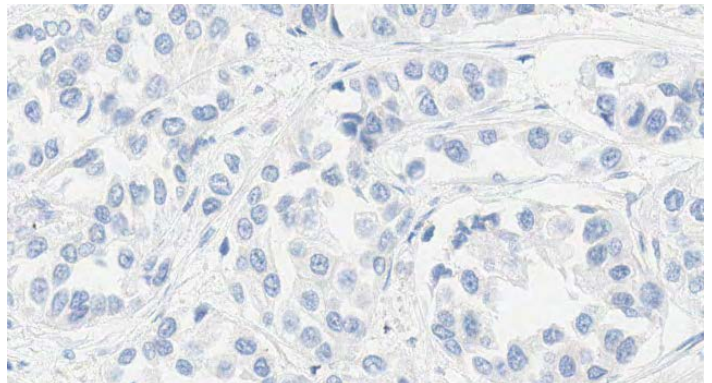
Lung squamous



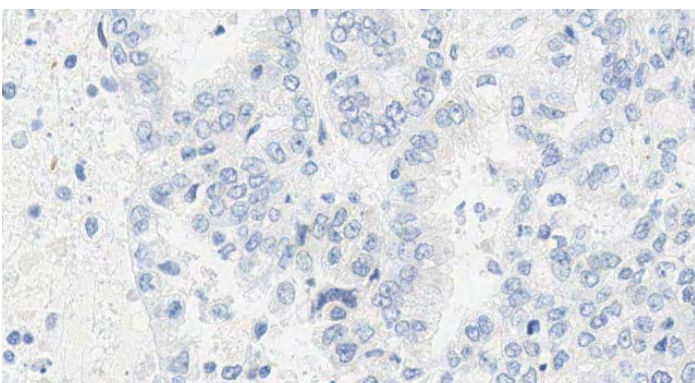
Endometrial cancer



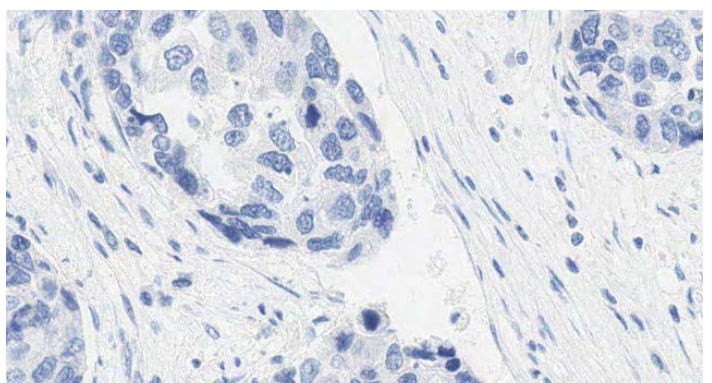
Bladder carcinoma



Ovarian carcinoma



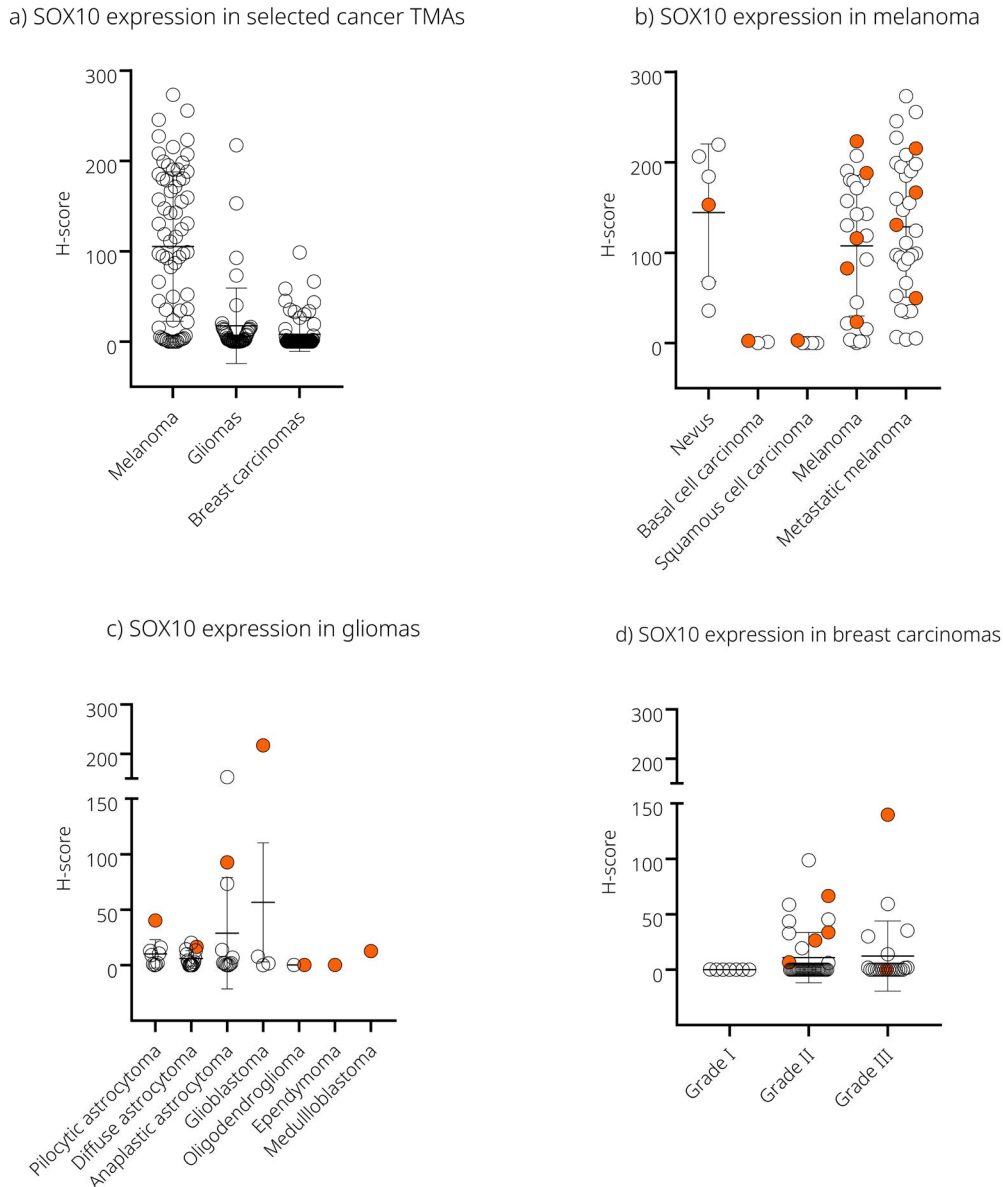
Isotype control (Breast fibroadenoma)



**Figure 4. SOX10 expression in cancer.** IHC staining of multi-cancer human tissues using anti-SOX10 (ab180682) 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.

## SOX10 expression in cancer (DISCOVERY ULTRA)

SOX10 expression varied in the analyzed cancer TMAs, with melanoma showing the highest H-score and breast cancers the lowest (a). The staining intensity of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD) (b-d).



**Figure 5. SOX10 protein expression in a selection of cancer TMAs.**

**(a)** Summary of the mean (with SD) H-score (maximum value 300) of SOX10 expression in TMA cores.

**(b)** H-score from 73 TMA cores/cases of melanoma (nevus (6), basal cell carcinoma (3), squamous cell carcinoma (5), melanoma (26), metastatic melanoma (33)).

**(c)** H-score averaged from 43 cases in duplicates (86 TMA cores) of gliomas (Pilocytic astrocytoma (9), Diffuse astrocytoma (15), Anaplastic astrocytoma (12), Glioblastoma (4), Oligodendroglioma (2), Ependymoma (1), Medulloblastoma (1)).

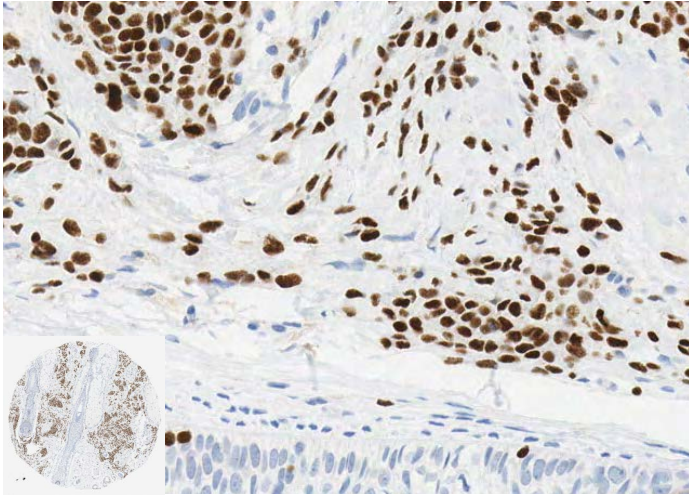
**(d)** SOX10 H-score from 70 cores/cases of breast cancer (IDC-infiltrative ductal carcinomas; grade I (1 & 1.5) (7), grade II (2 & 2.5) (40), grade III (23)).

**(b-d)** The IHC images corresponding to orange data points are shown in Figures 6-8.

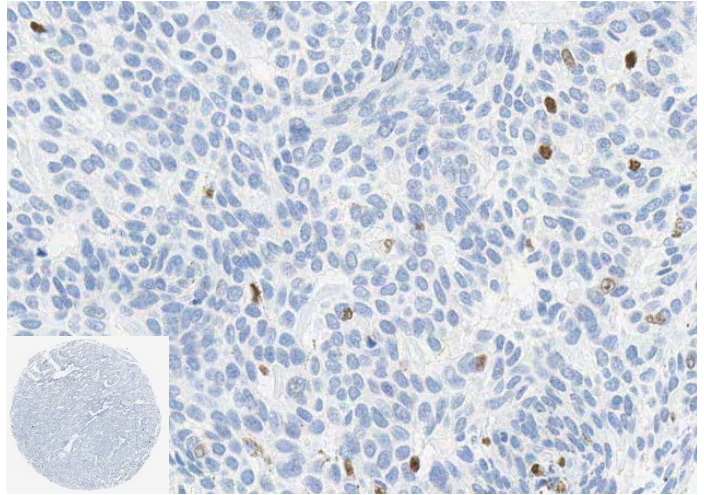
## SOX10 expression in melanoma TMA (DISCOVERY ULTRA)

Below are the representative images of human melanoma TMA showing weak to strong SOX10 expression.

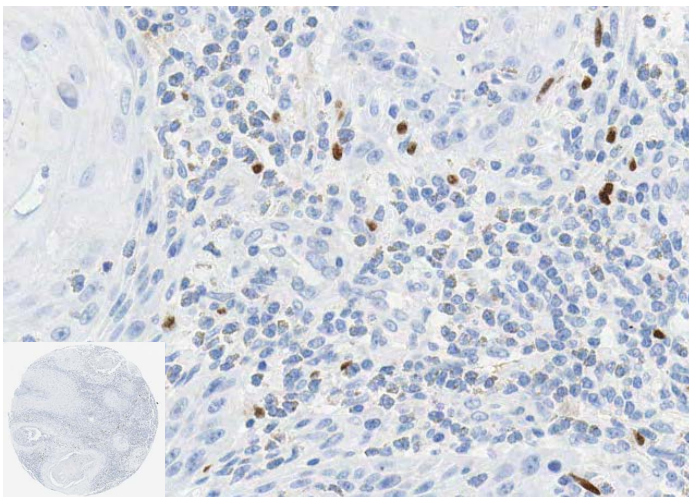
**Nevus**  
(H score 153.14)



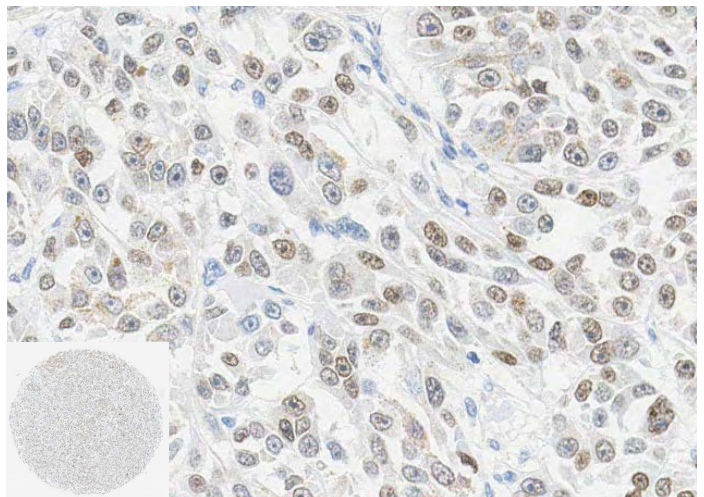
**Basal cell carcinoma**  
(H score 2.48)



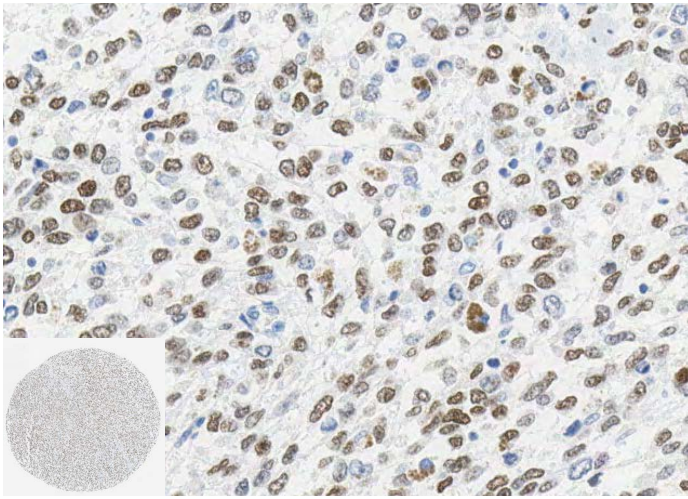
**Squamous cell carcinoma**  
(H score 3.02)



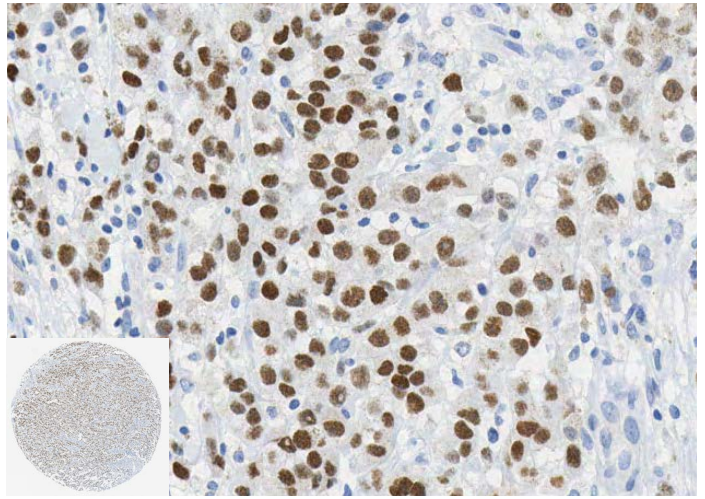
**Melanoma**  
(H score 23.53)



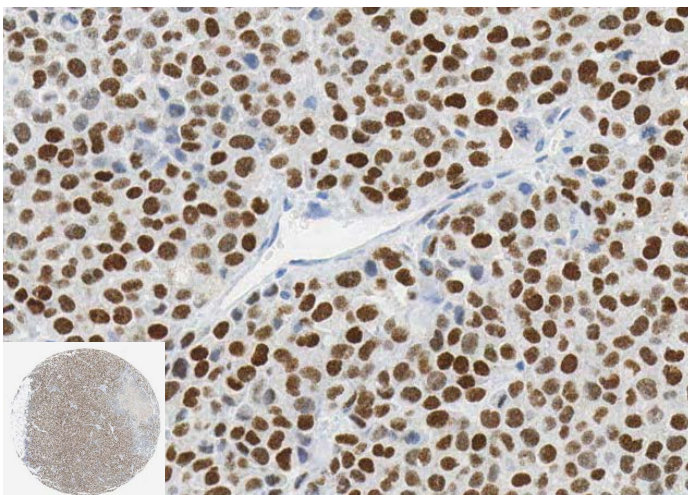
**Melanoma**  
(H score 82.51)



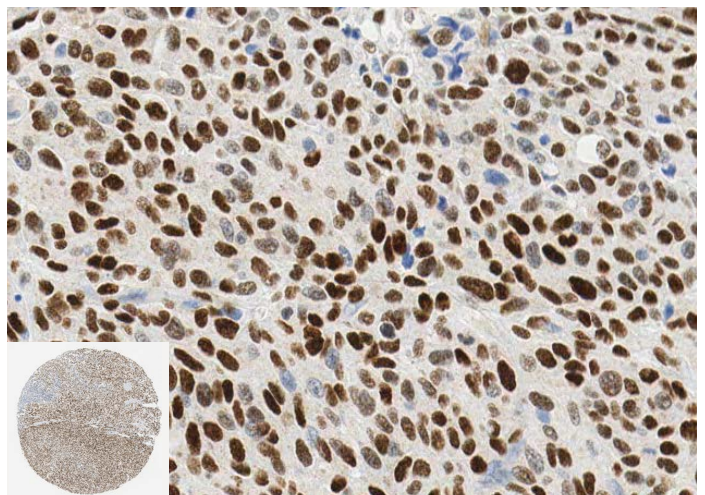
**Melanoma**  
(H score 115.88)



**Melanoma**  
(H score 188.22)

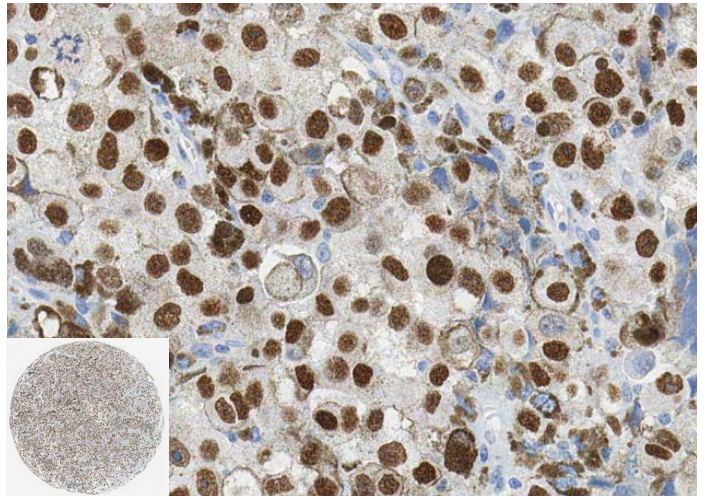
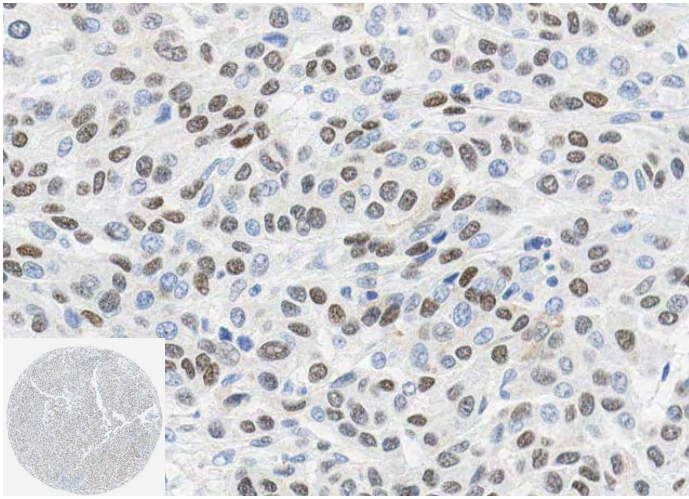


**Melanoma**  
(H score 223.26)



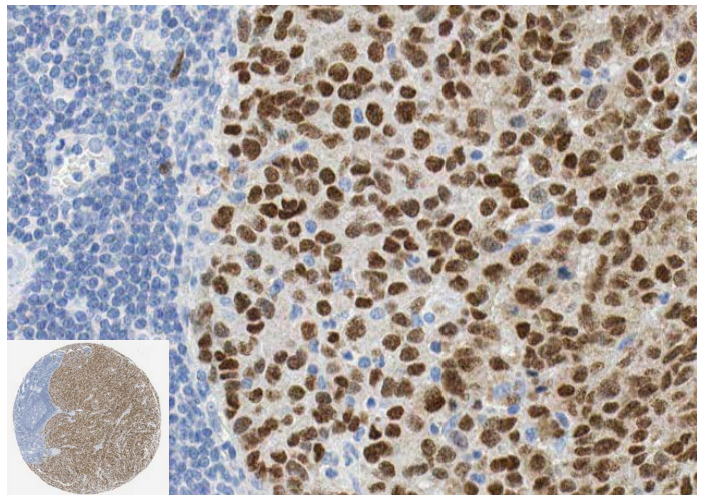
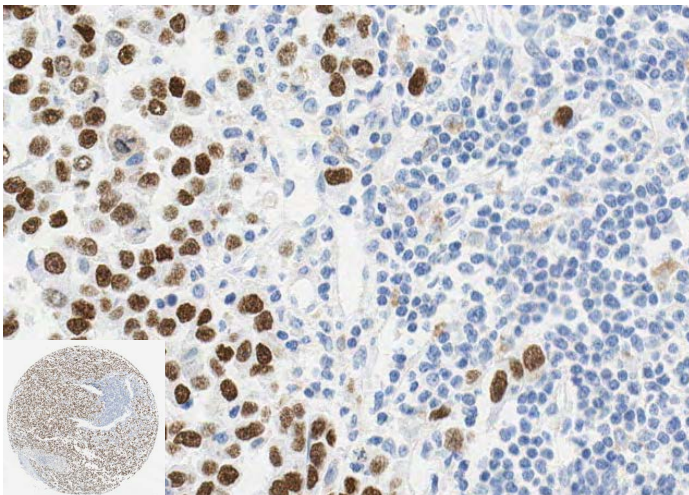
**Metastatic melanoma**  
(H score 49.82)

**Metastatic melanoma**  
(H score 130.76)



**Metastatic melanoma**  
(H score 166.64)

**Metastatic melanoma**  
(H score 215.43)

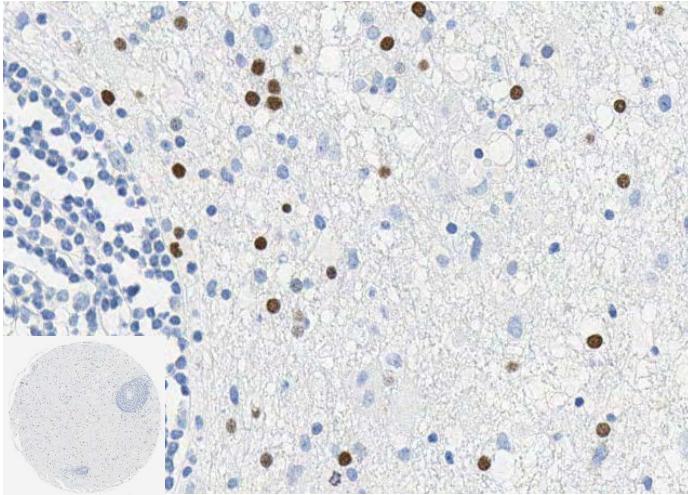


**Figure 6: SOX10 expression in melanoma.** IHC images show weak, moderate or strong SOX10 staining in brown (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

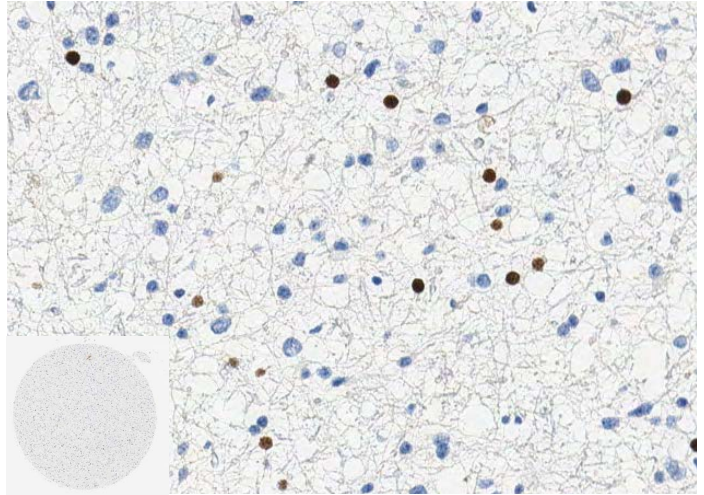
## SOX10 expression in glioma TMA (DISCOVERY ULTRA)

Below are the representative images of human glioma TMA showing weak to strong SOX10 expression.

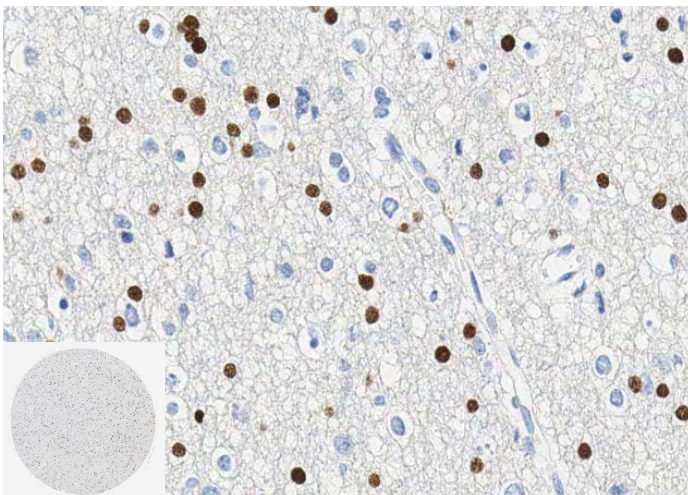
**Pilocytic astrocytoma**  
(H score 40.35)



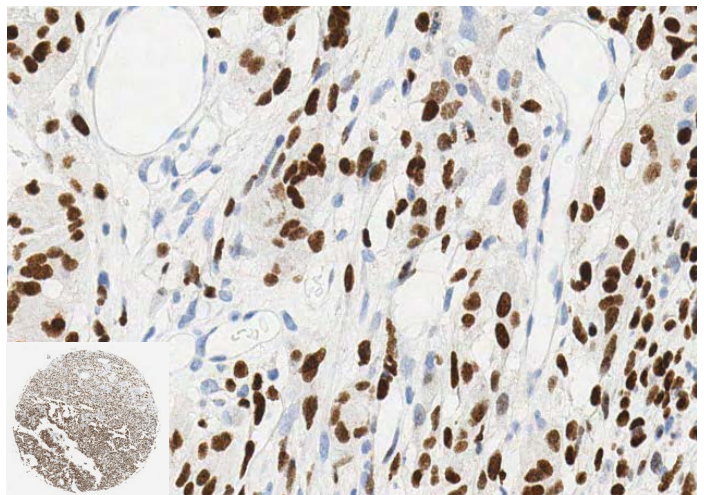
**Diffuse astrocytoma**  
(H score 16.49)



**Anaplastic astrocytoma**  
(H score 92.77)

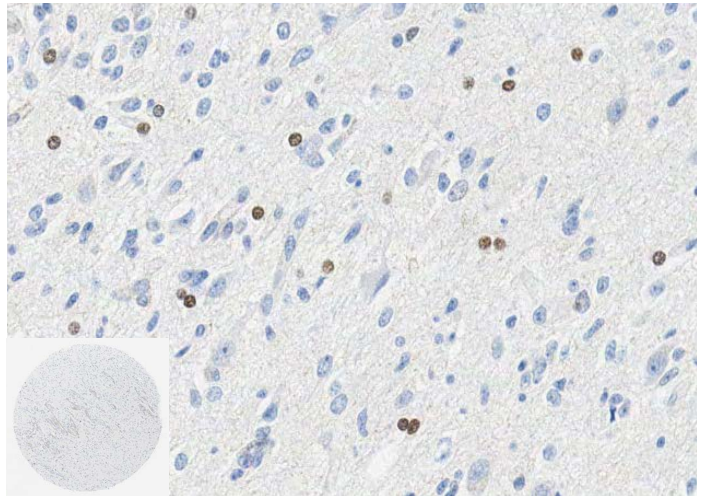
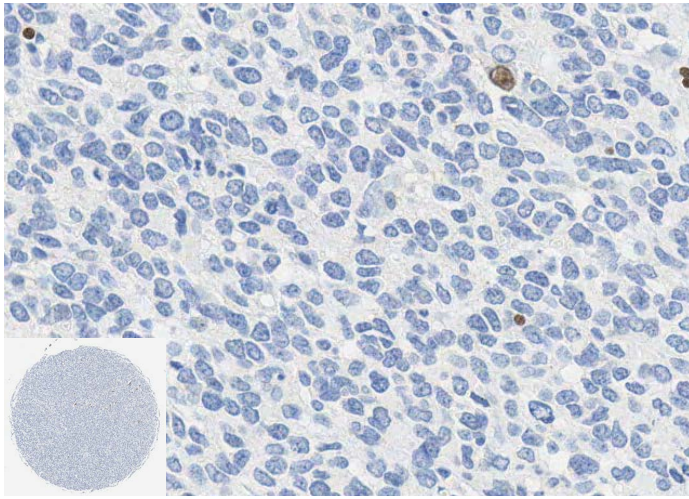


**Glioblastoma**  
(H score 217.22)



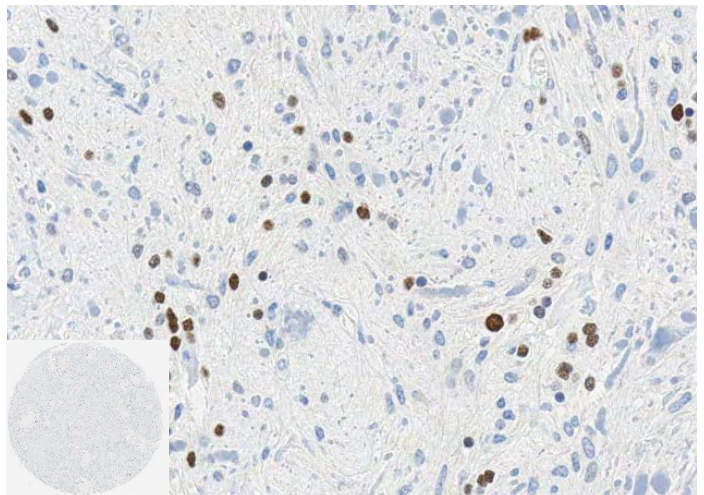
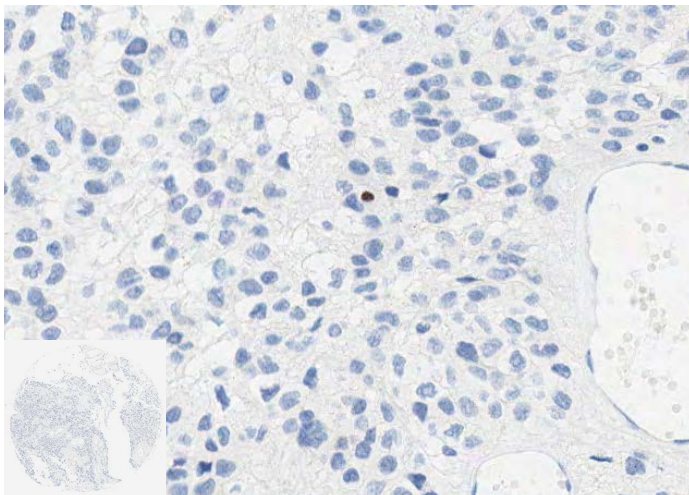
**Glioblastoma**  
(H score 1.66)

**Oligodendroglioma**  
(H score 0.30)



**Ependymoma**  
(H score 0.23)

**Medulloblastoma**  
(H score 12.78)

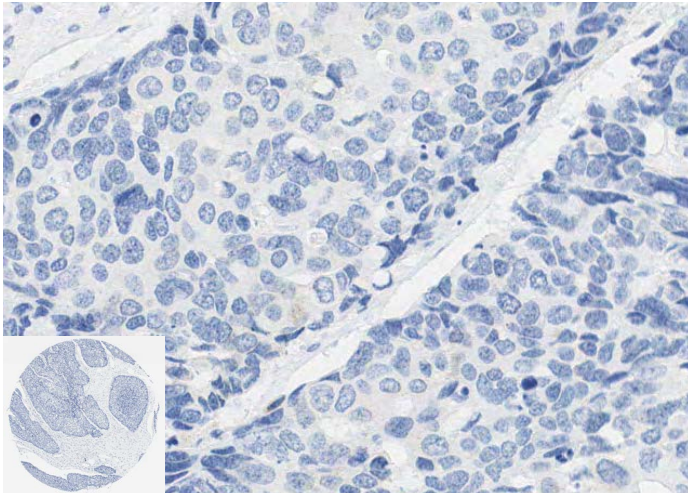


**Figure 7: SOX10 expression in gliomas.** IHC images show weak, moderate or strong SOX10 staining in brown (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

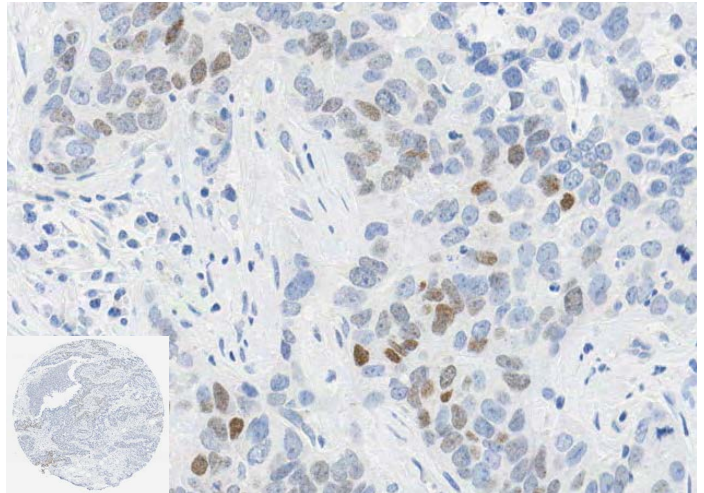
## SOX10 expression in breast cancer TMA (DISCOVERY ULTRA)

Below are the representative images of human breast cancer (Invasive ductal carcinoma (IDC)) TMA showing weak to strong SOX10 expression. Grade I comprises both grade 1 and 1.5, and grade II comprises both grade 2 and 2.5.

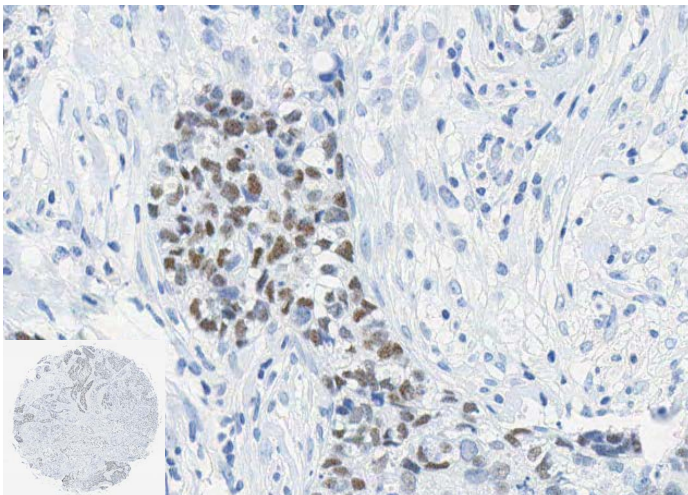
**IDC Grade III**  
(H score 0.00)



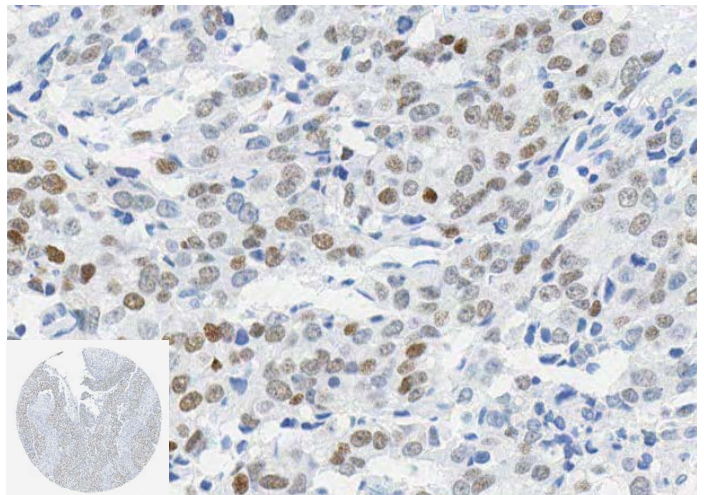
**IDC Grade II**  
(H score 6.67)



**IDC Grade II**  
(H score 26.44)

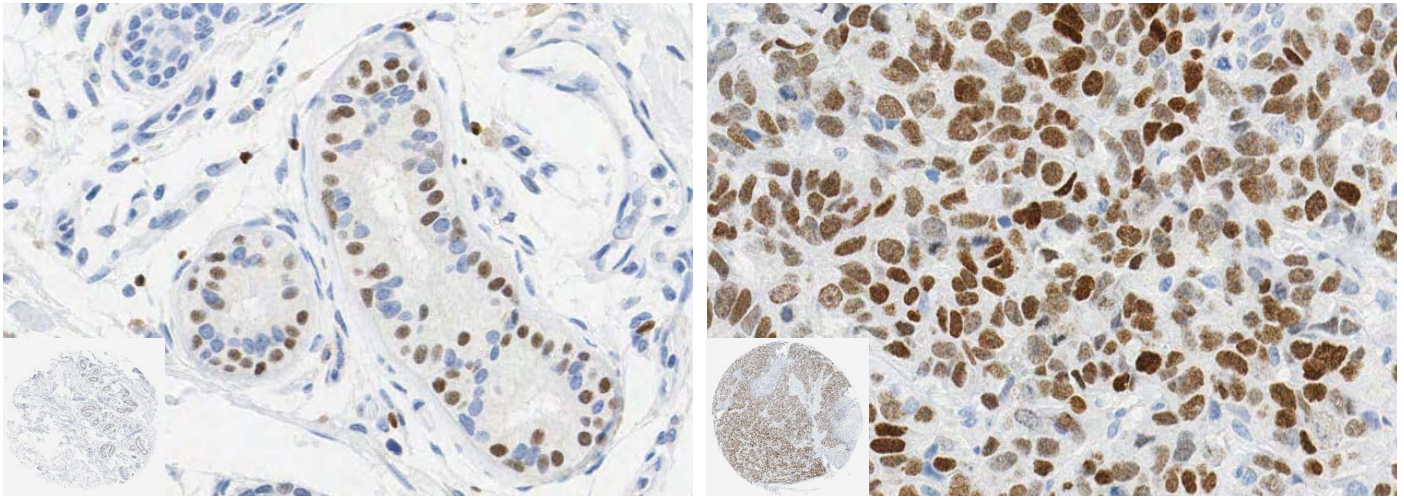


**IDC Grade II**  
(H score 33.78)



**IDC Grade II**  
(H score 66.44)

**IDC Grade III**  
(H score 139.85)



**Figure 8: SOX10 expression in breast cancer.** IHC images show weak, moderate or strong SOX10 staining in brown (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x (whole core insets at 5x) on the Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

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