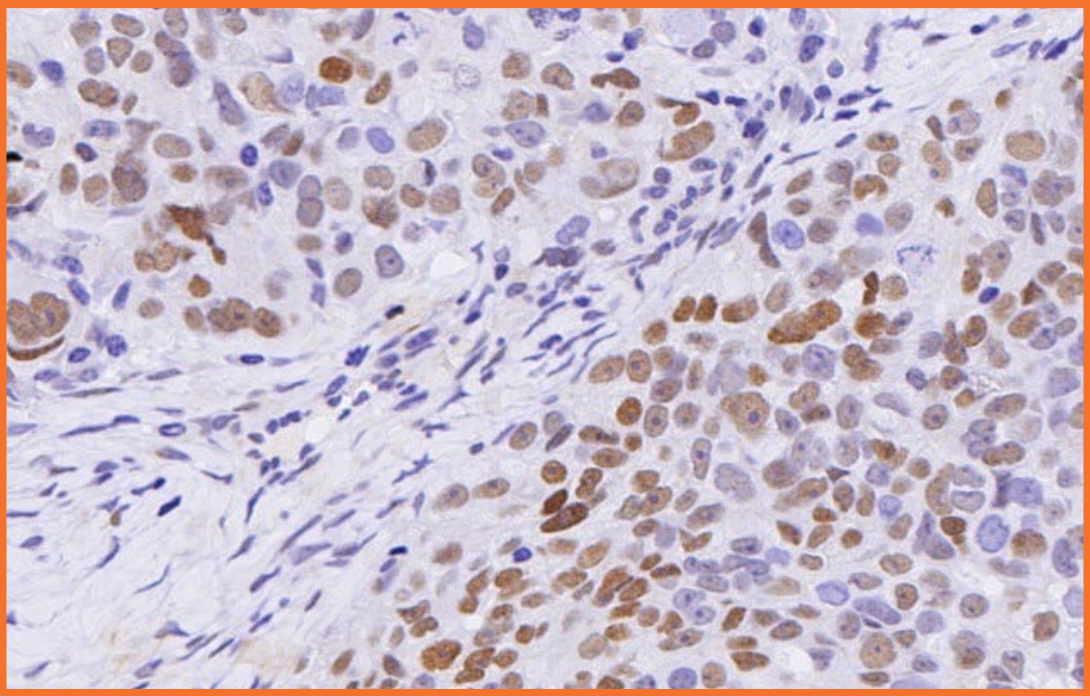
Enhanced validation data

Anti-c-Myc antibody [Y69] - ChIP Grade- recombinant antibody – ab32072



c-Myc expression in serous ovarian cystadenocarcinoma

AB0453_v1.0_090225

progress happens together

abcam

Enhanced validation of Anti-c-Myc antibody [Y69] - ChIP Grade – ab32072

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 and 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-c-Myc antibody (ab32072) in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®) and DISCOVERY ULTRA system (Roche Diagnostics).

— A quantitative H-score analysis of c-Myc expression was performed using the artificial intelligence (AI)-driven digital image analysis software Visiopharm® (Visiopharm A/S).

AB0453_v1.0_090225

Target overview

HGNC symbol
MYC

Approved name
MYC proto-oncogene, bHLH transcription factor

Chromosomal location
8q24.21

Function

- c-Myc is a key regulator of metabolism and cell growth, influencing many oncogenic pathways and driving gene expression changes that promote cancerous transformation[1-4].

Tissue specificity

- c-Myc is expressed in various normal tissues, and elevated expression levels have been observed in several cancers.

Cellular localization

- Nucleus

Database links

Entrez Gene: 4609
OMIM®: 190080
Uniprot: P01106

AB0453_v1.0_090225

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[5-8].

Tissue microarray (TMA)	Cores	Cases	Normal/ Benign cases	Cancer cases	Source (#catalog number)
Multi-normal (a)	15	15	15	0	In-house TMA
Multi-cancer (b)	35	35	1	34	In-house TMA
Ovarian cancer	102	102	7	95	Pantomics (#OVC1021)
Stomach cancer	102	102	5	97	Pantomics (#STC1021)
Prostate cancer	102	102	5	97	Pantomics (#PRC1021)

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 a single donor: colon, cerebrum, tonsil, stomach, testis, prostate, lung, skeletal muscle, heart, skin, spleen, pancreas, kidney, placenta, and liver.

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, 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 CC2)	32 min, 100 °C
Post cell conditioning enzyme	Protease III	4 min
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Anti-c-Myc antibody [Y69]- ab32072 diluted in VENTANA Antibody Diluent with Casein (#760-219) to final concentration of 0.5 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).

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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	Time (minutes)	Number of washes
Peroxide block	3-4% (v/v) Hydrogen peroxide	5 min	-
Wash	Bond™ wash solution	0 min	3x
Primary antibody	Anti-c-Myc antibody [Y69]- ab32072 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 0.5 µg/mL	15 min	
Wash	Bond™ wash solution	0 min	4x
Secondary antibody	Bond™ polymer refine detection (DS9800)	8 min	
Wash	Bond™ wash solution	4 min	2x
	Deionized water	0 min	1x
Visualization	Mixed DAB refine (DS9800)	0 min	1x
	Mixed DAB refine (DS9800)	10 min	
Wash	Deionized water	0 min	3x
Counterstain	Hematoxylin (DS9800)	5 min	
Wash	Deionized water	0 min	1x
	Bond™ wash solution	0 min	1x
	Deionized water	0 min	1x

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.

H-score analysis

A quantitative H-score analysis of c-Myc expression 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.

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 whole core in the TMAs were calculated in Visiopharm®, and the graphical representation was generated using GraphPad Prism 10.

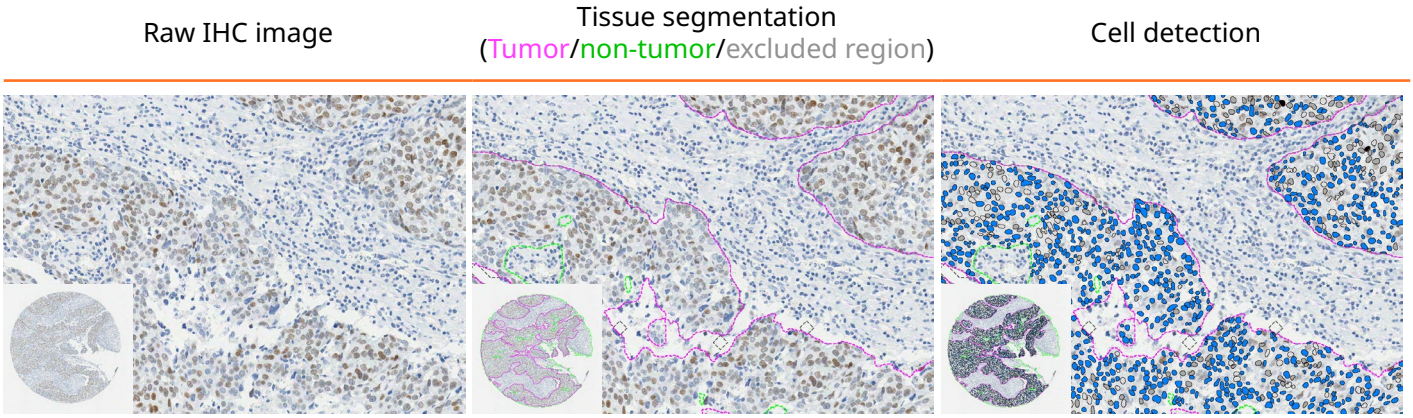


Figure 1. Raw IHC images were subjected to total cell detection to determine staining intensity. There are four intensity scores shown here: blue (0), white (1+), grey (2+), and black (3+).

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-score = [(0x % of negative cells) + (1x % of weak stained cells) + (2x % of moderate stained cells) + (3x % of strong stained cells), giving an analytical range from 0 to 300.

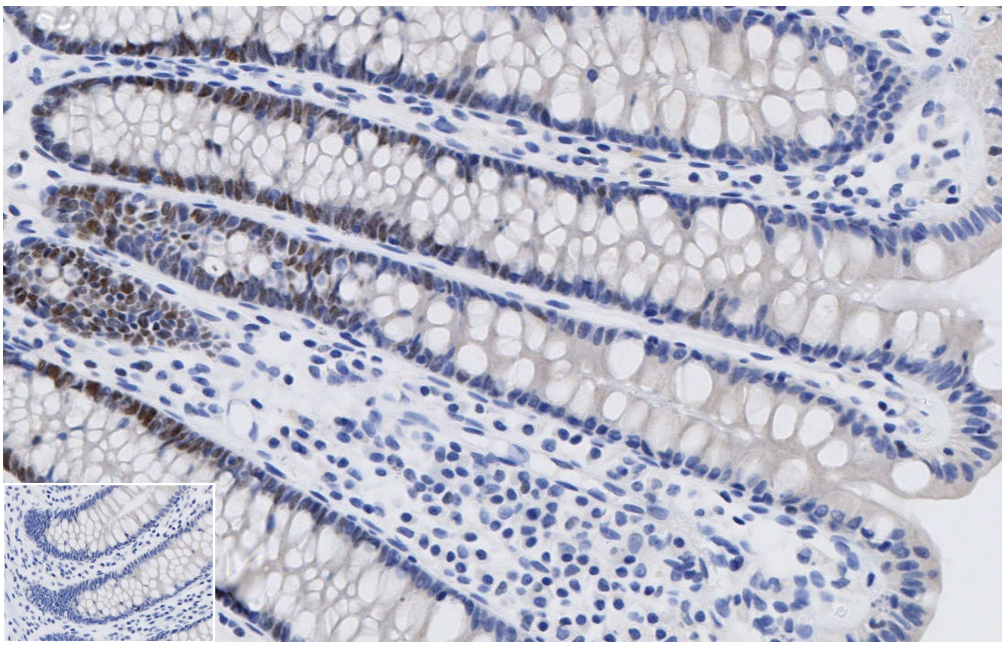
AB0453_v1.0_090225

c-Myc expression in multi-normal TMA (DISCOVERY ULTRA)

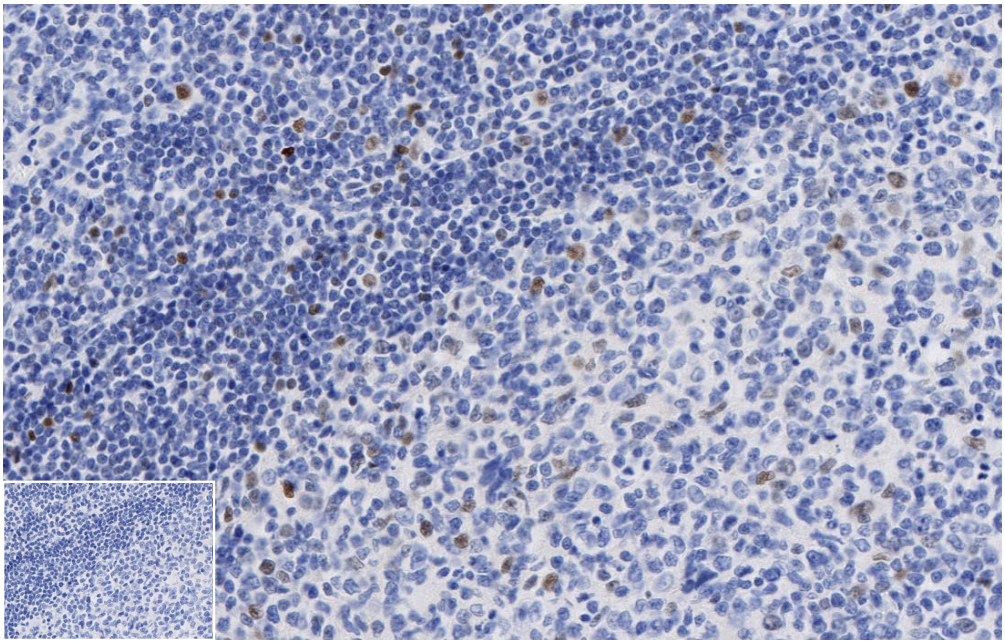
Below are representative images of selected tissues from multi-normal TMA. c-Myc expression was detected in the colon, tonsil, skin, spleen, and stomach, whereas low to no expression was observed in the brain, testis, and skeletal muscle tissues.

c-Myc

Colon



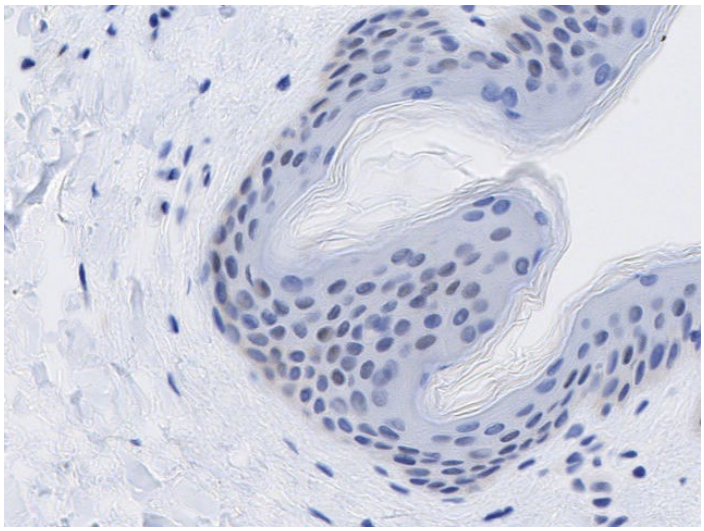
Tonsil



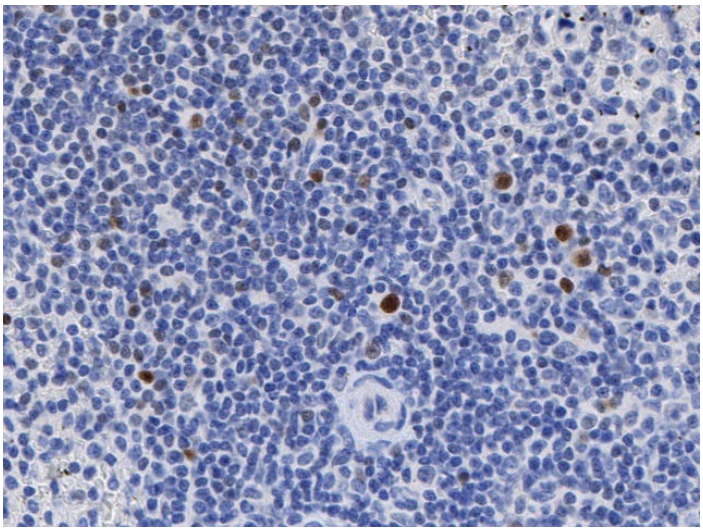
Enhanced validation data

c-Myc

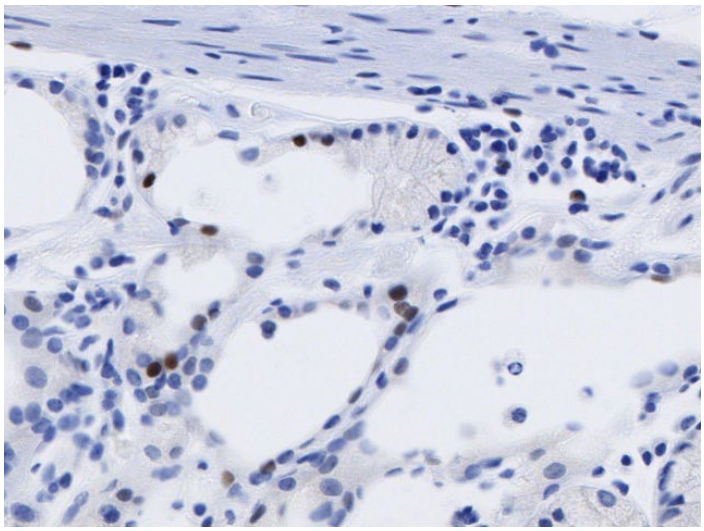
Skin



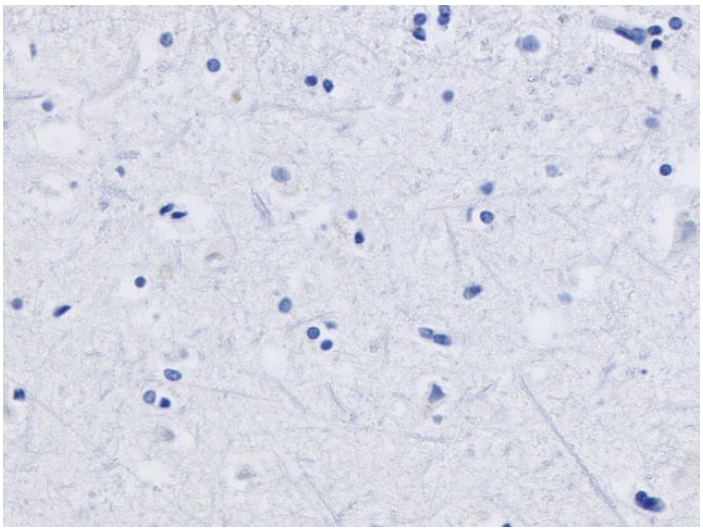
Spleen



Stomach



Cerebrum



c-Myc

Testis	Skeletal muscle

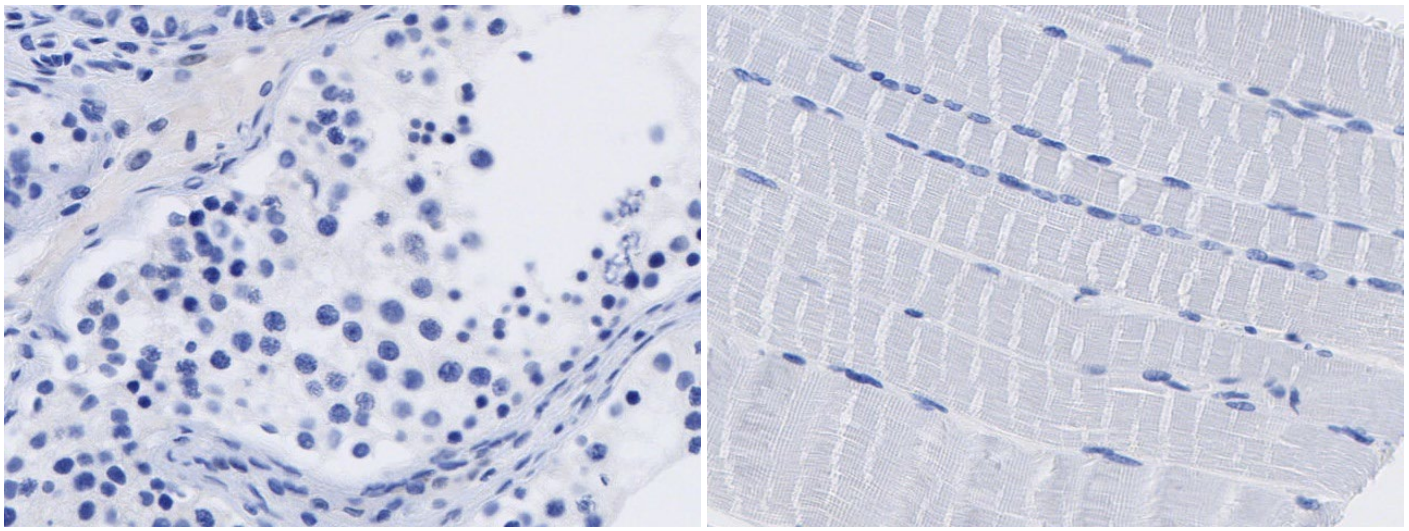


Figure 2. c-Myc expression in human normal tissue. IHC staining of multi-normal human tissues using anti-c-Myc (ab32072) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

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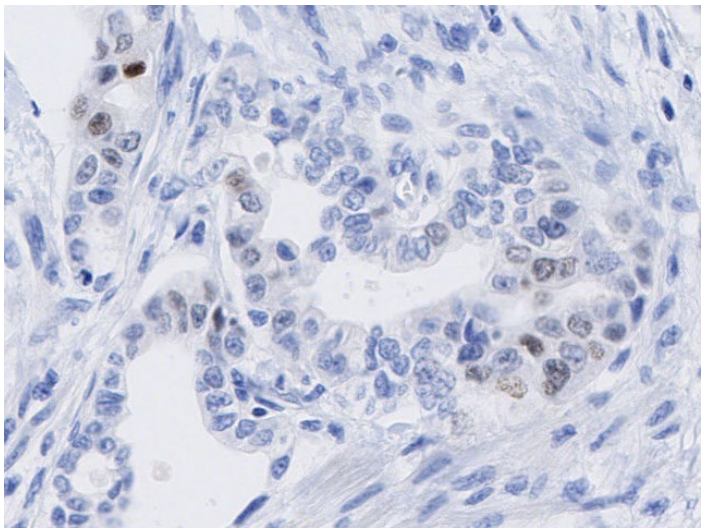
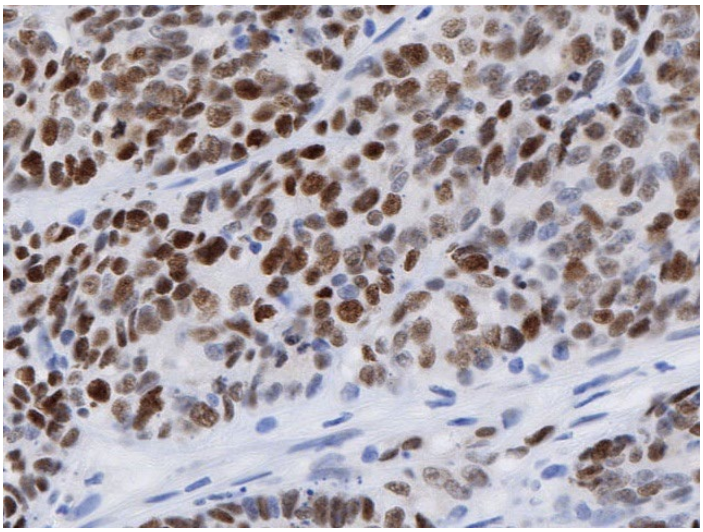
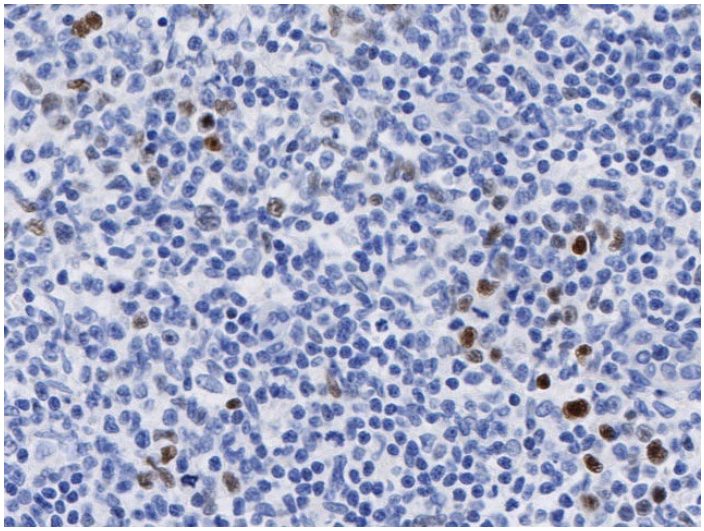
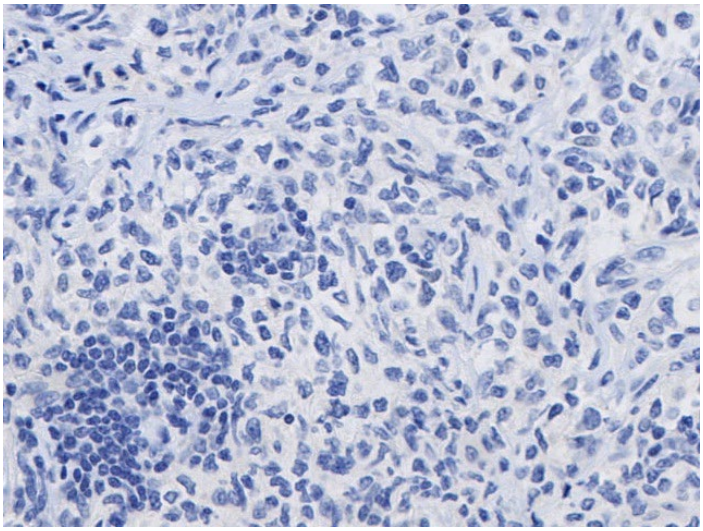
c-Myc expression in multi-cancer TMA (DISCOVERY ULTRA)

Below are the representative images of selected tissues from the multi-cancer TMA.
c-Myc expression was detected in adenocarcinoma of the stomach, colon, pancreas, T cell lymphoma, ovarian cancer, seminoma, glioblastoma, and cervix. No expression was observed in B cell lymphoma, renal cell carcinoma, and endometrial cancer.

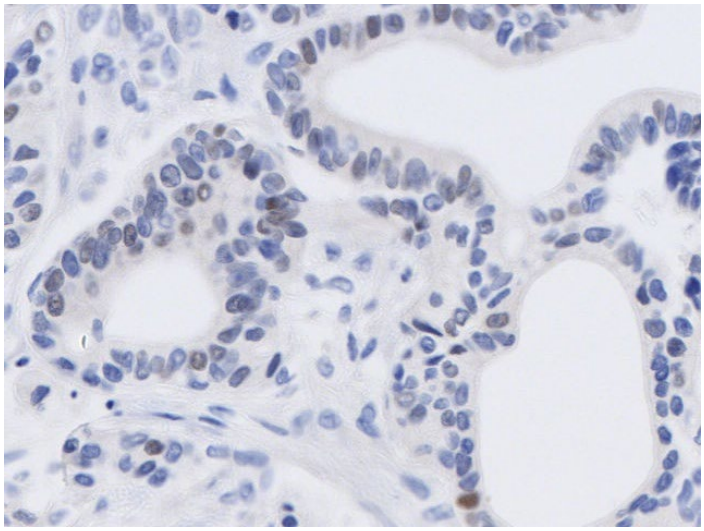
c-Myc

Stomach adenocarcinoma	Invasive colon adenocarcinoma
	
T cell lymphoma	B cell lymphoma
	

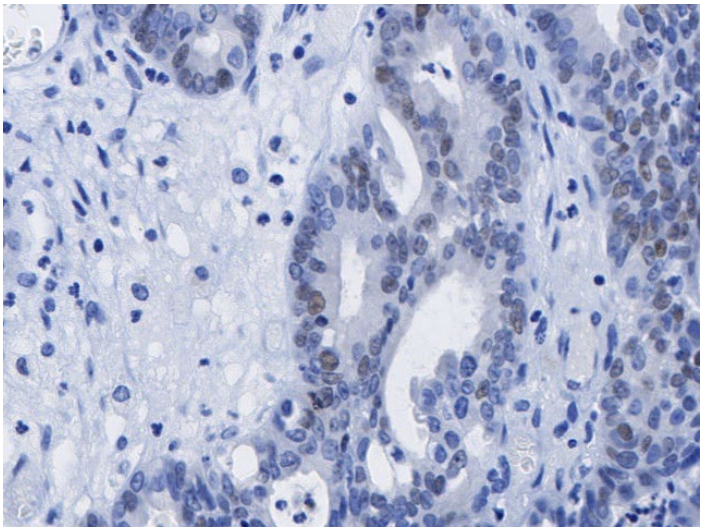
Enhanced validation data

c-Myc

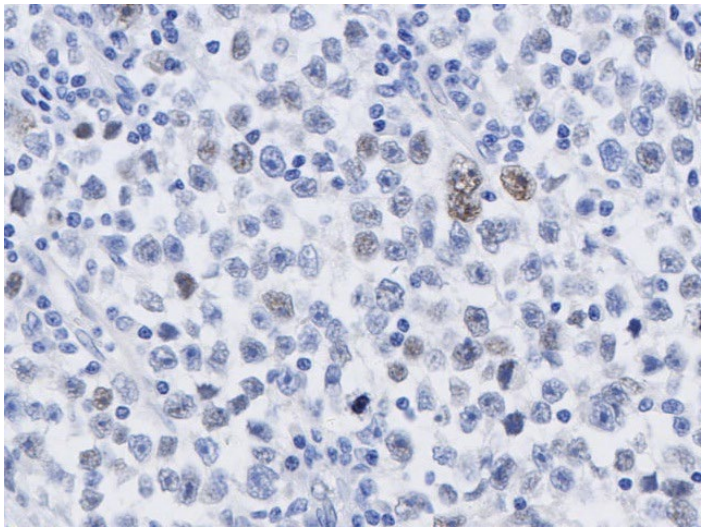
Pancreas carcinoma



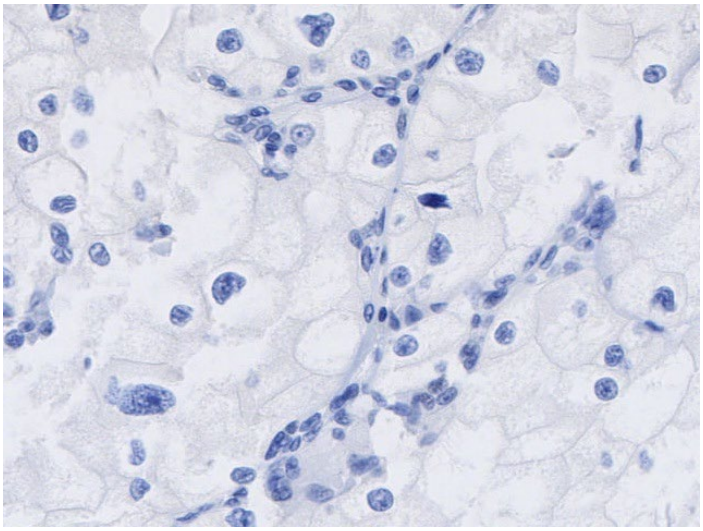
Ovarian cancer



Seminoma

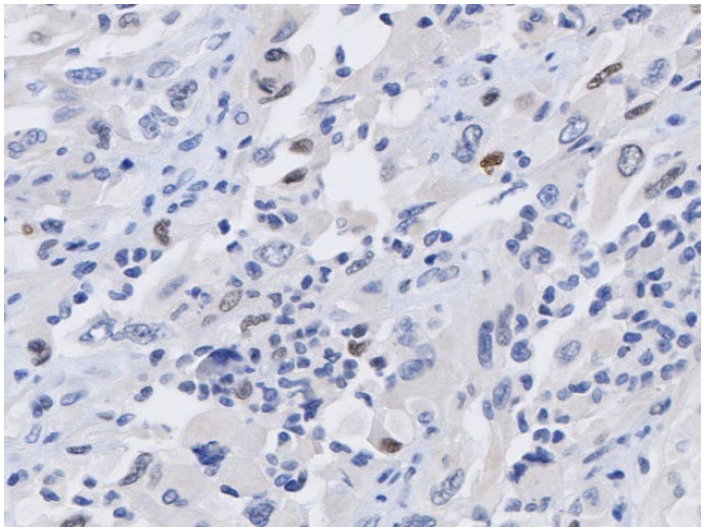


Renal cell carcinoma

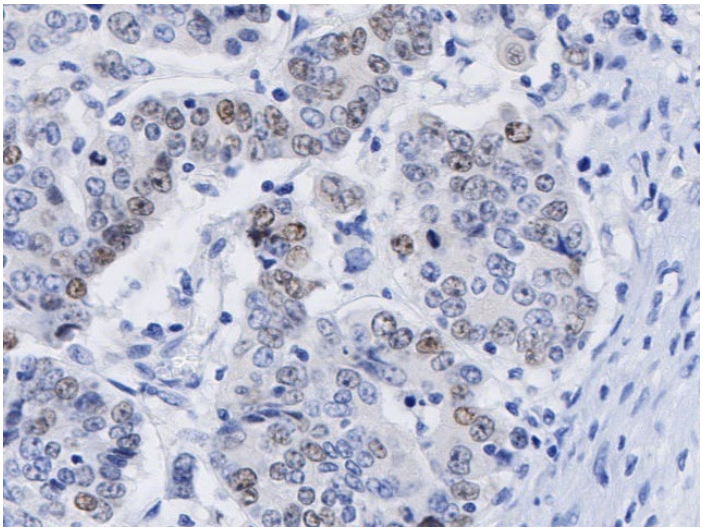


c-Myc

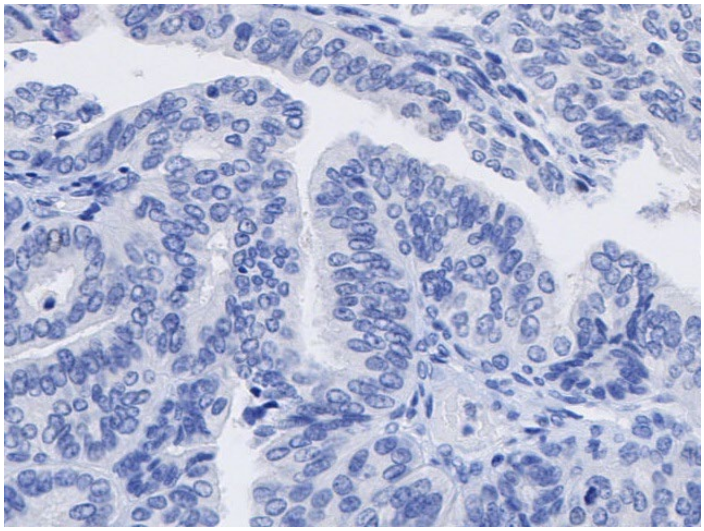
Glioblastoma



Cervical cancer



Endometrial cancer



Isotype control - Invasive colon adenocarcinoma

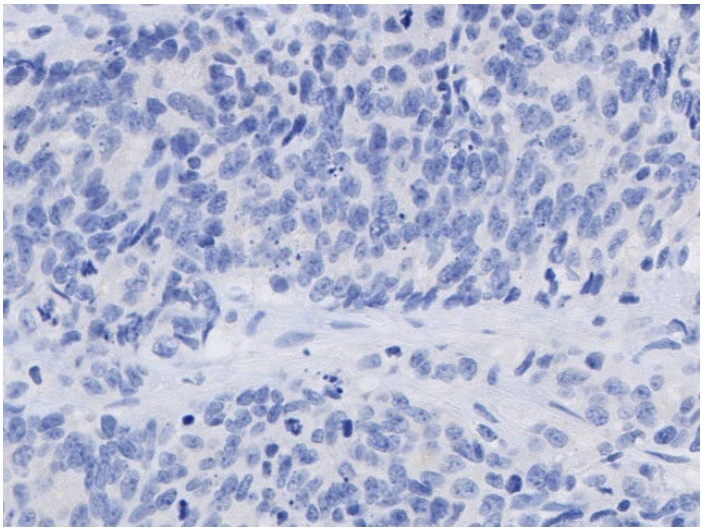


Figure 3. c-Myc expression in cancer. IHC staining of multi-cancer human tissues using anti-c-Myc (ab32072) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

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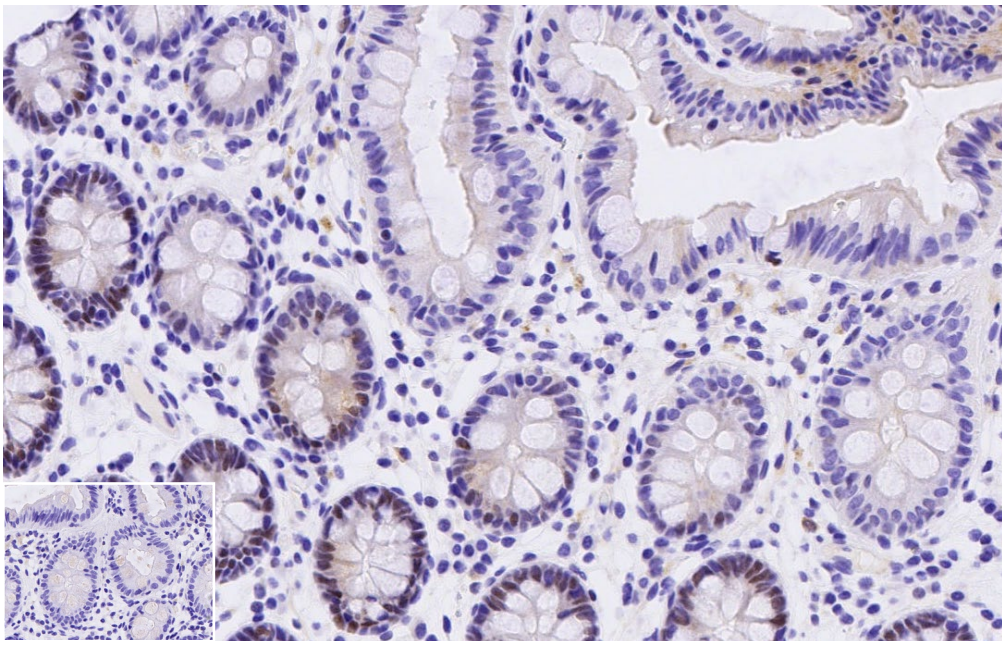
c-Myc expression in multi-normal TMA (BOND™ RX)

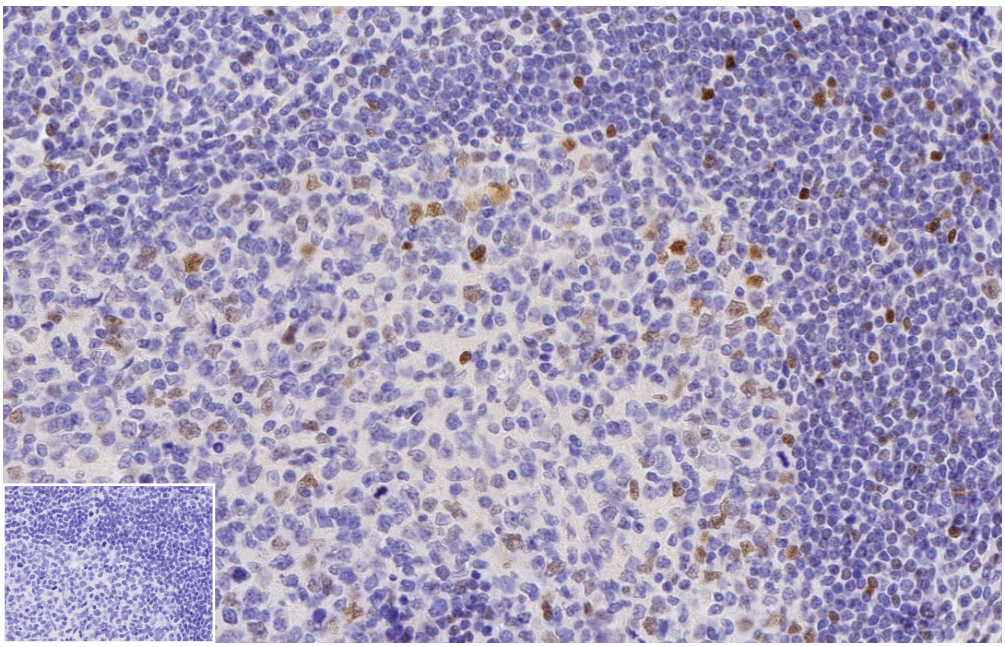
Below are the representative images of selected tissues from the multi-normal TMA. c-Myc expression was detected in the colon, tonsil, skin, spleen, and stomach. Low to no expression was observed in the brain, testis, and skeletal muscle tissues.

c-Myc

Colon



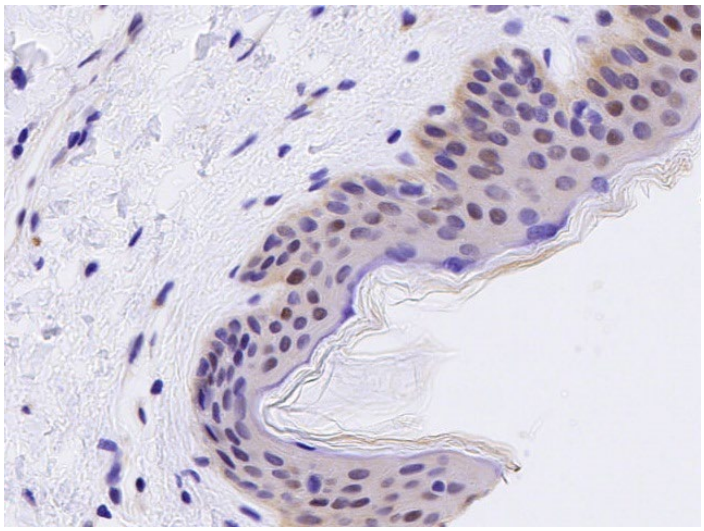
Tonsil



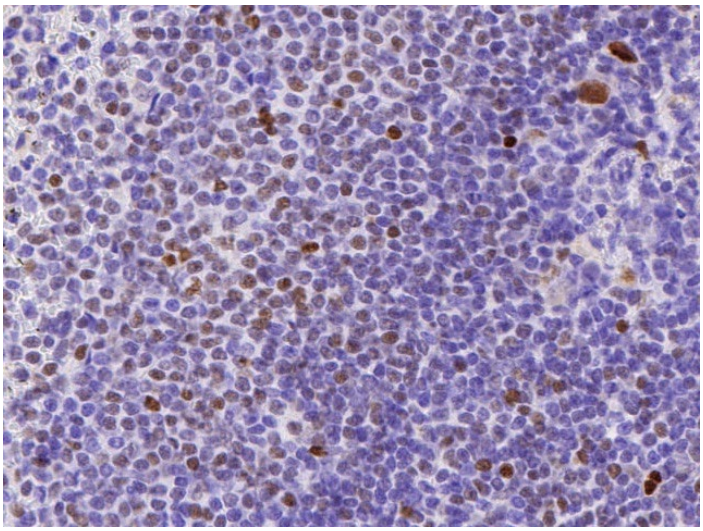
Enhanced validation data

c-Myc

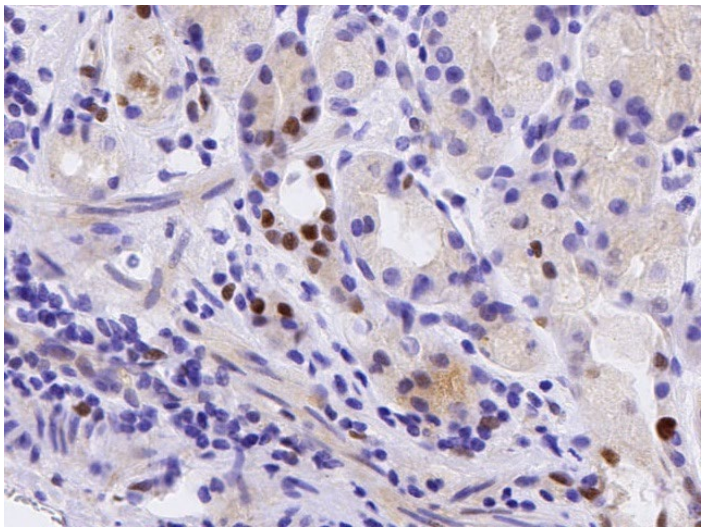
Skin



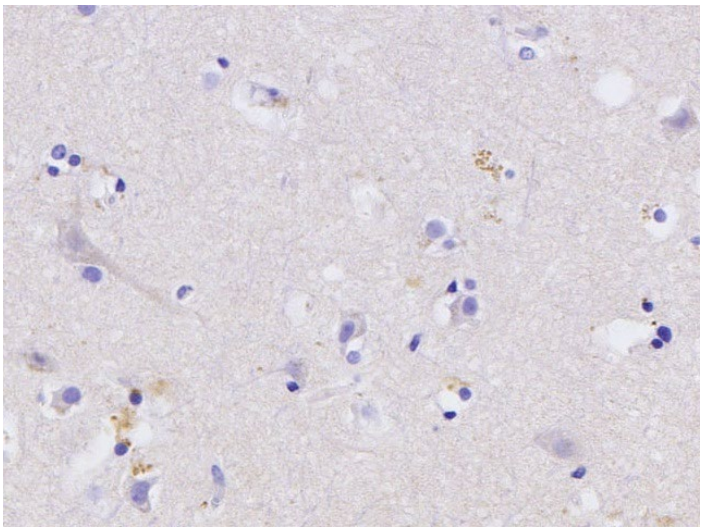
Spleen



Stomach



Cerebrum



c-Myc

Testis	Skeletal muscle

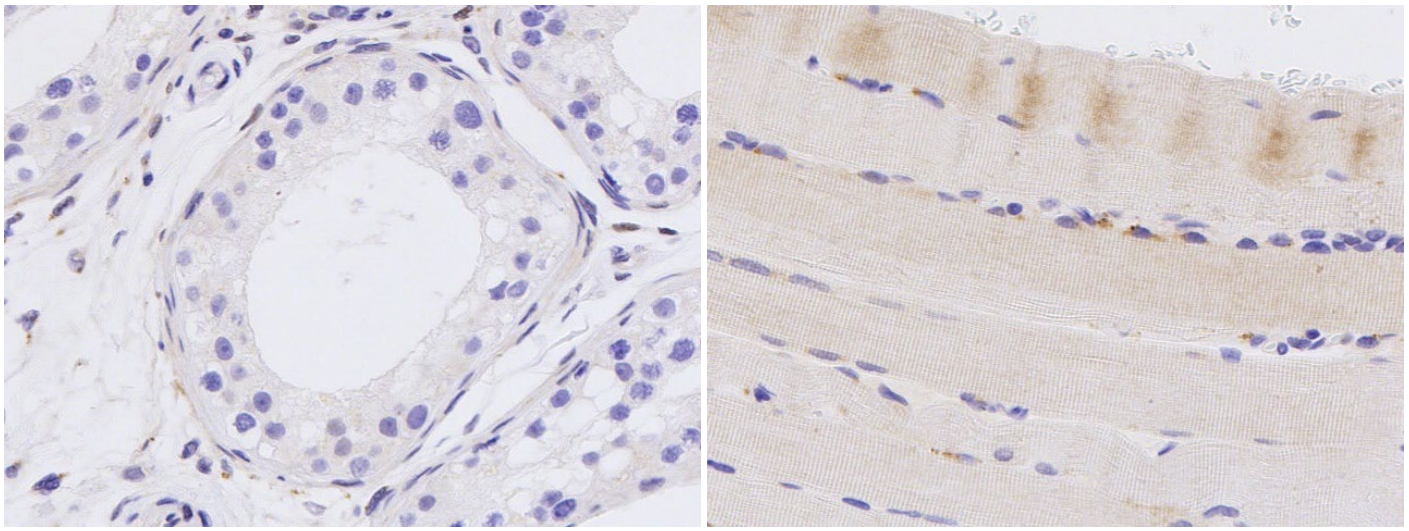


Figure 4. c-Myc expression in human normal tissue. IHC staining of multi-normal human tissues using anti-c-Myc (ab32072) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

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c-Myc expression in multi-cancer TMA (BOND™ RX)

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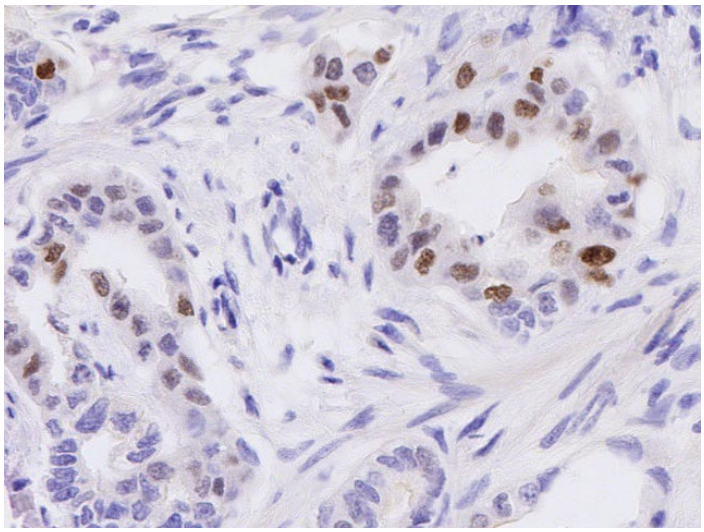
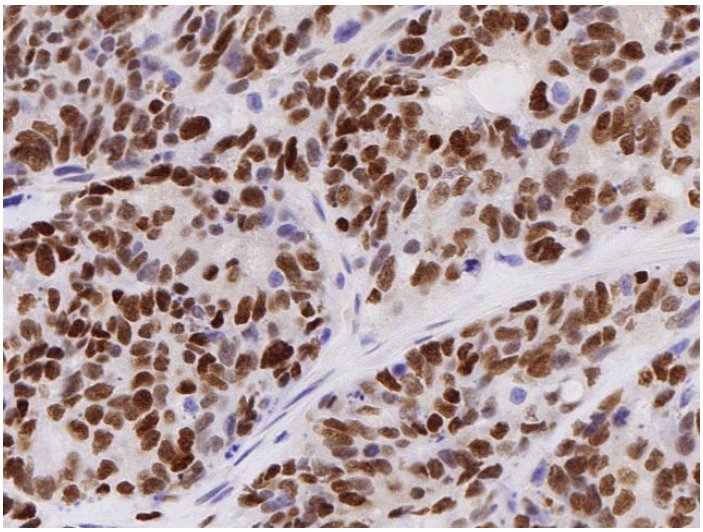
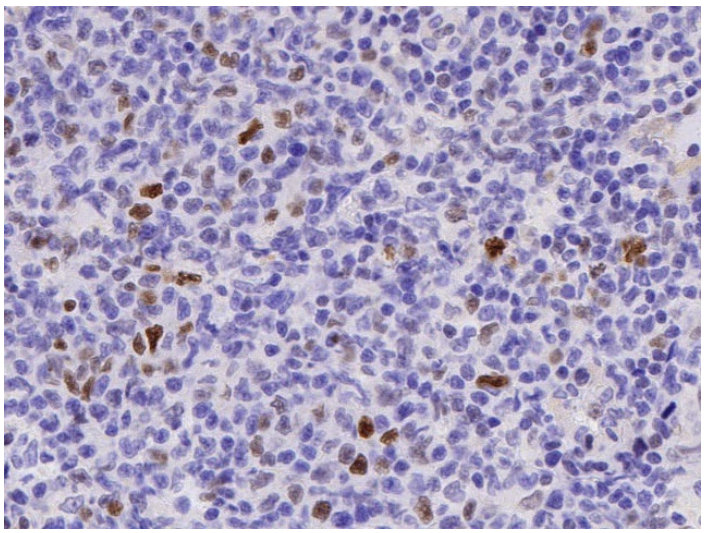
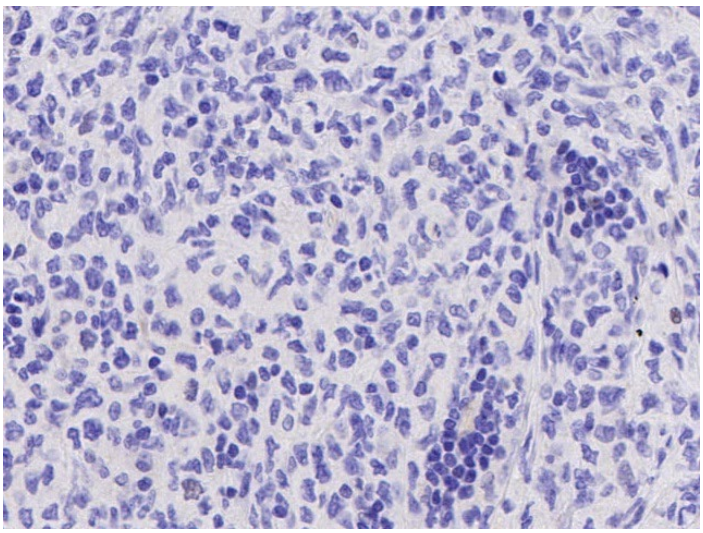
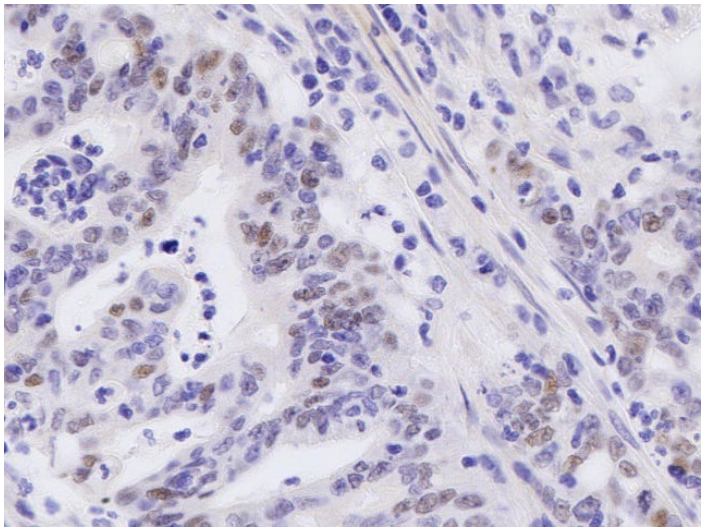
c-Myc

Stomach adenocarcinoma	Invasive colon adenocarcinoma
	
T cell lymphoma	B cell lymphoma
	

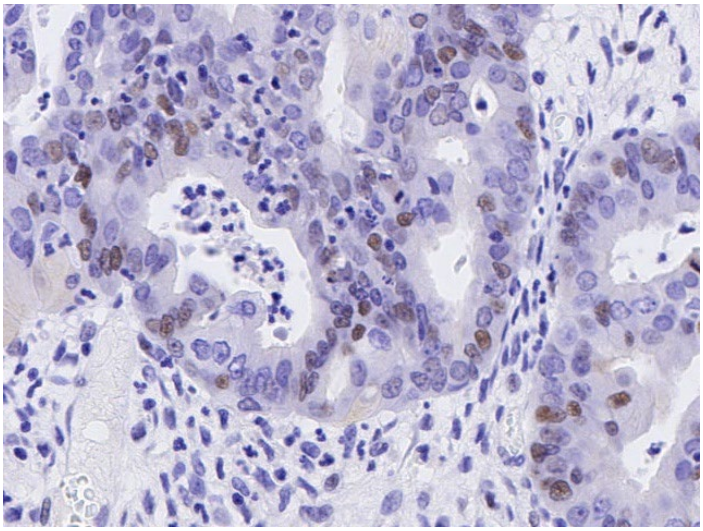
Enhanced validation data

c-Myc

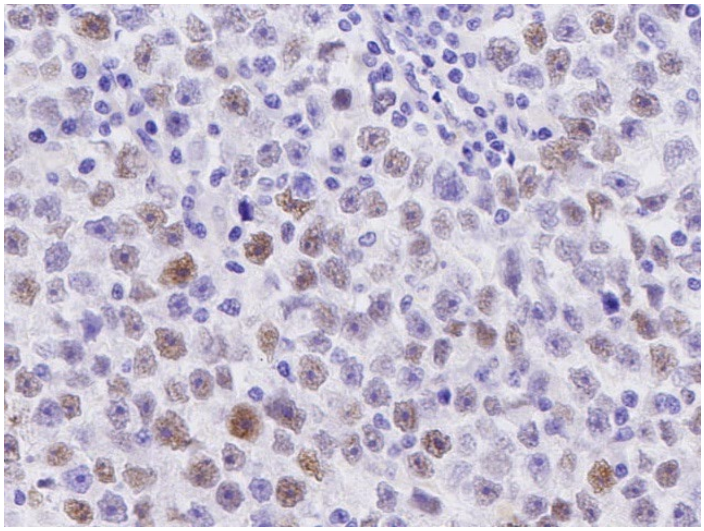
Pancreas adenocarcinoma



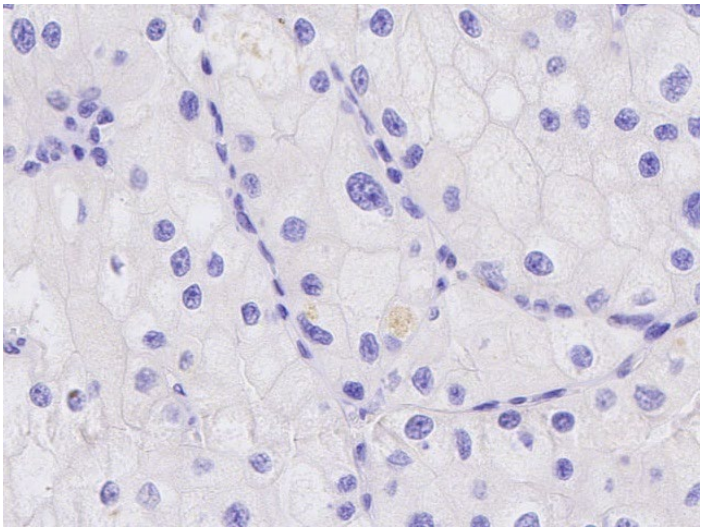
Ovarian cancer



Seminoma

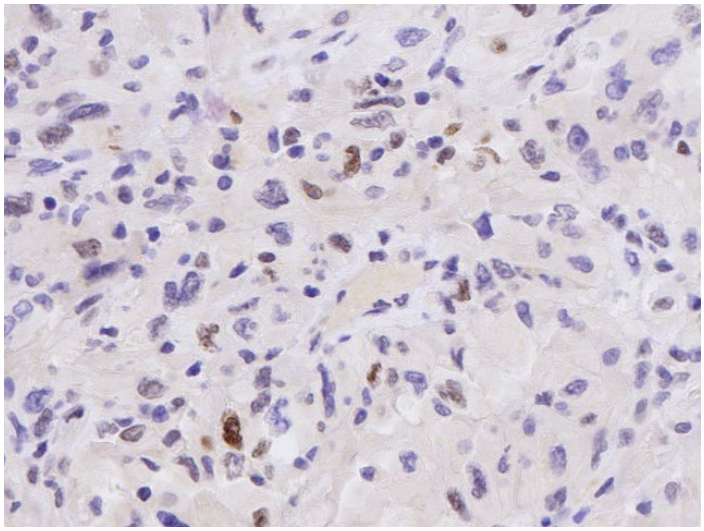


Renal cell carcinoma

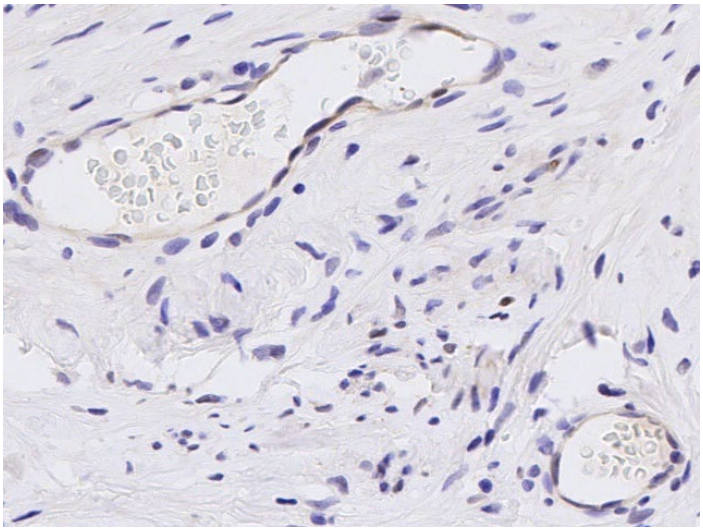


c-Myc

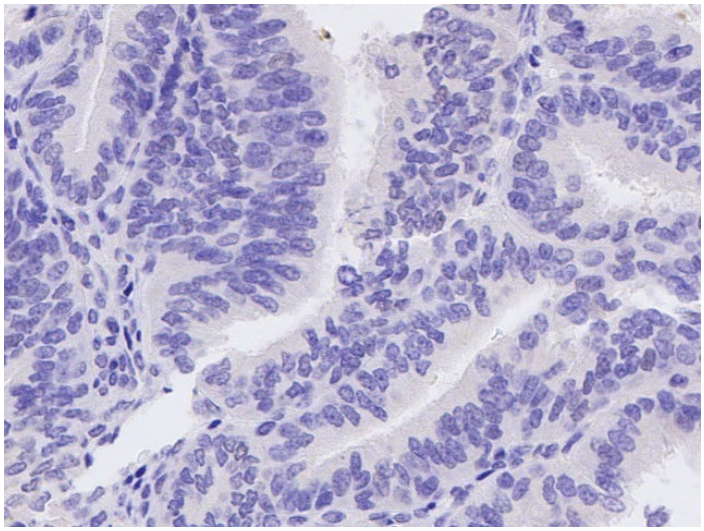
Glioblastoma



Cervical cancer



Endometrial cancer



Isotype control - Invasive colon adenocarcinoma

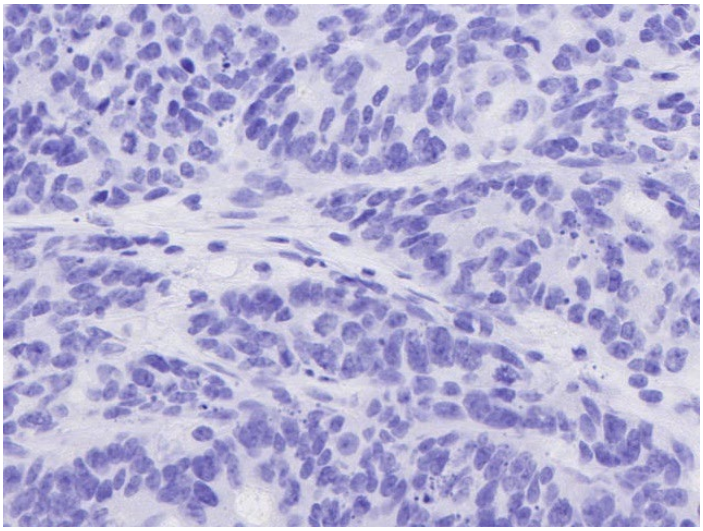


Figure 5. c-Myc expression in cancer. IHC staining of multi-cancer human tissues using anti- c-Myc (ab32072) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

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c-Myc expression in cancer (BOND™ RX)

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

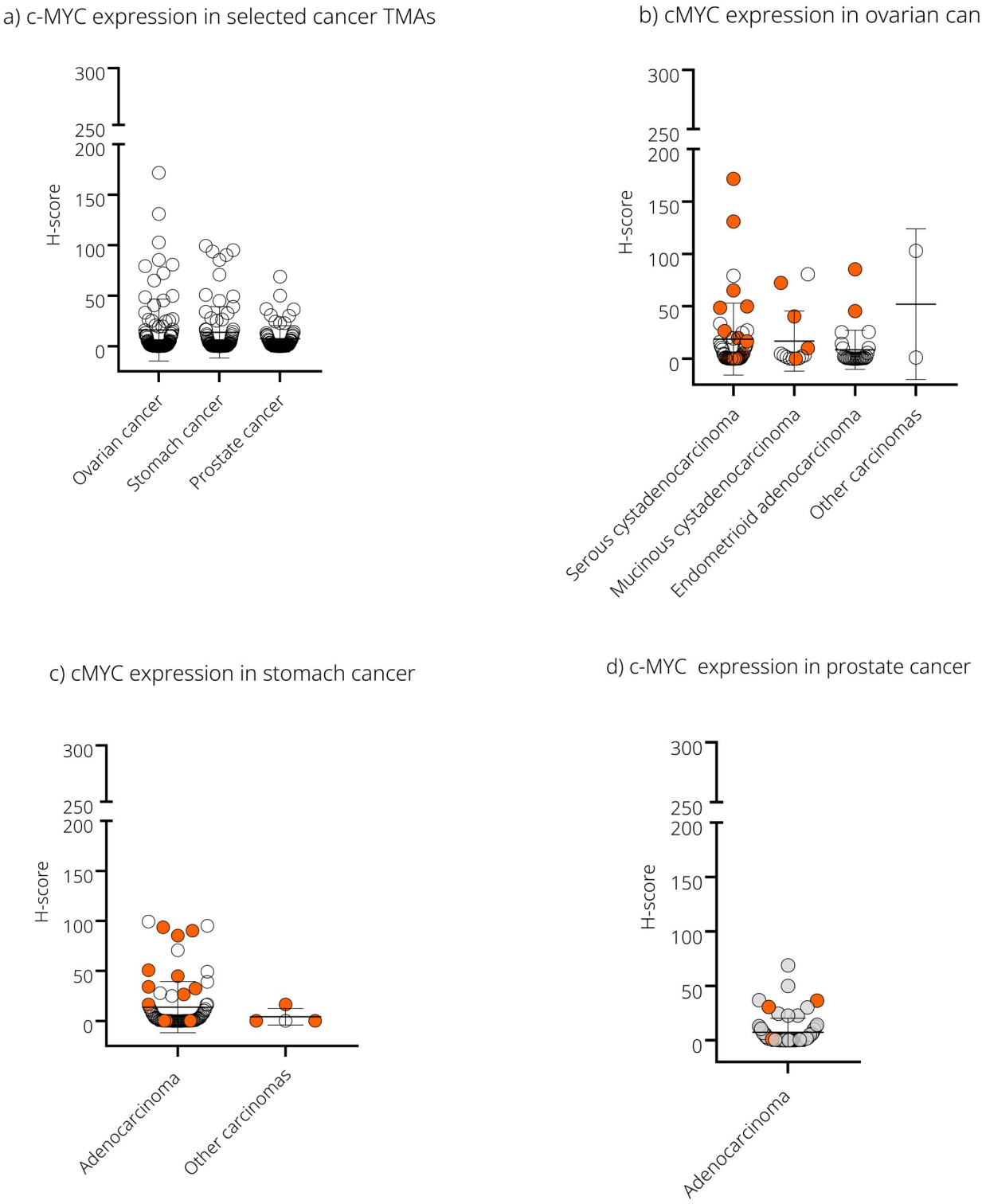


Figure 6. c-Myc protein expression in a selection of cancer TMAs.

Enhanced validation data

a. The scatter plot (with SD) summarizes the relative average DAB intensity of c-Myc expression in selected cancer TMA cores (ovarian cancer (87), stomach cancer (76) and prostate cancer (67))

b. H-score from 87 TMA cores/cases of ovarian cancer (Serous cystadenocarcinoma (45), Mucinous cystadenocarcinoma (13), Endometrioid adenocarcinoma (27) and other carcinoma (Borederline mixed cystadenoma and Clear cell carcinoma (2)). The IHC images corresponding to orange data points are shown in Figure 7.

c. H-score from 76 TMA cores/cases of stomach cancer (Adenocarcinoma (72), and other carcinoma (Mucinous adenocarcinoma and Undifferentiated carcinoma (4)). The IHC images corresponding to orange data points are shown in Figure 8.

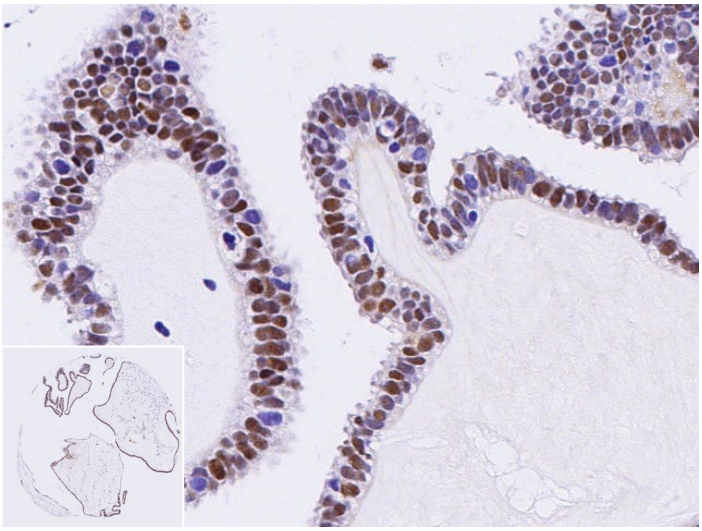
d. H-score averaged from 67 TMA cores/cases of prostate adenocarcinoma (72). The IHC images corresponding to orange data points are shown in Figure 9.

c-Myc expression in ovarian cancer TMA (BOND™ RX)

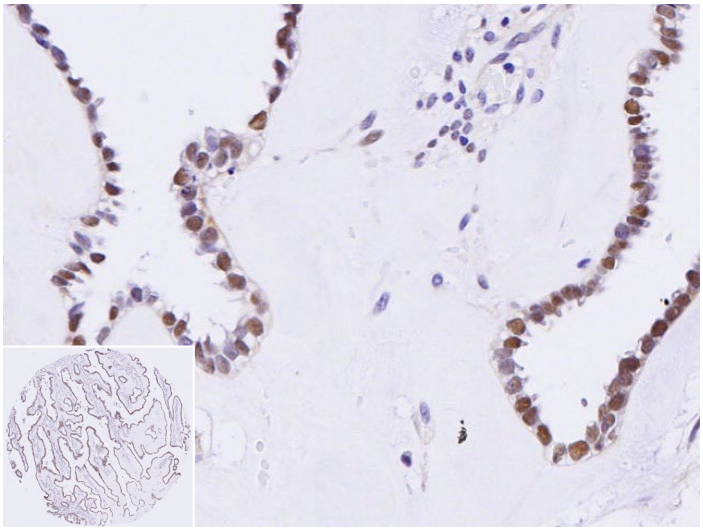
Below are the representative images of human ovarian cancer TMA showing strong to weak c-Myc expression.

c-Myc

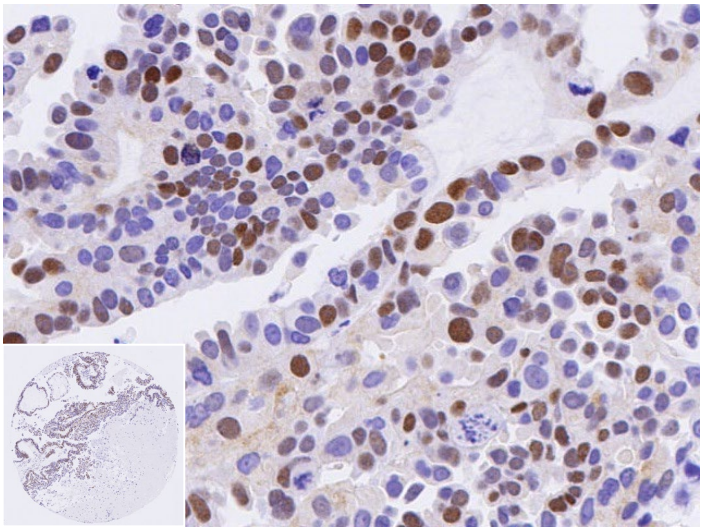
Serous cystadenocarcinoma (171.59)



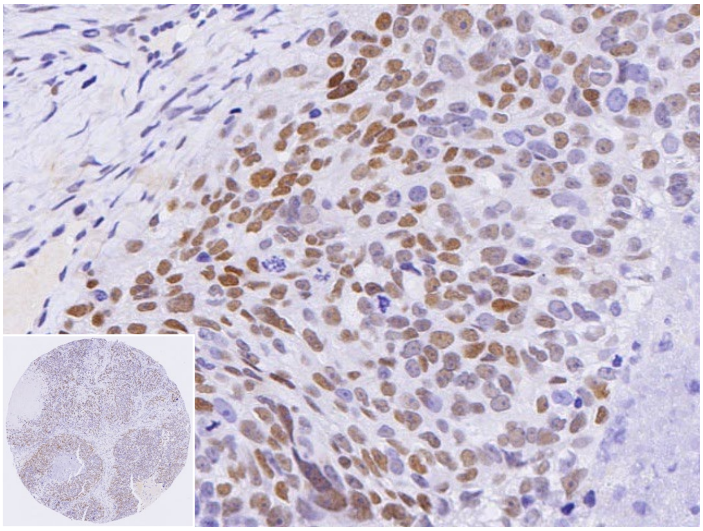
Serous cystadenocarcinoma (130.92)



Serous cystadenocarcinoma (65.01)



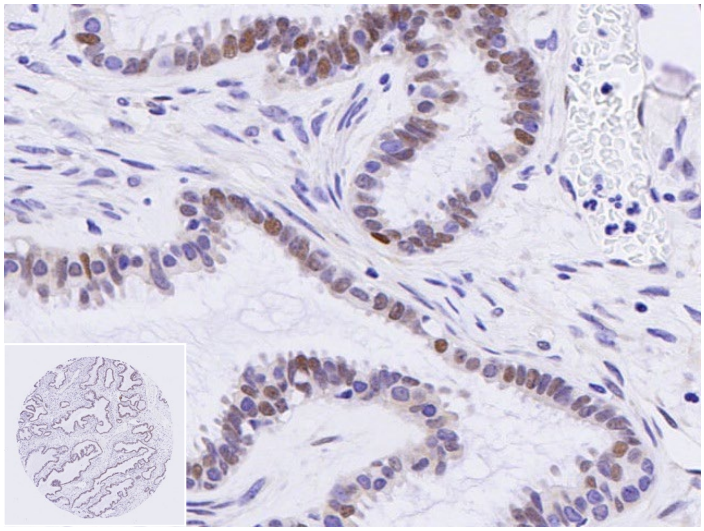
Serous cystadenocarcinoma (49.80)



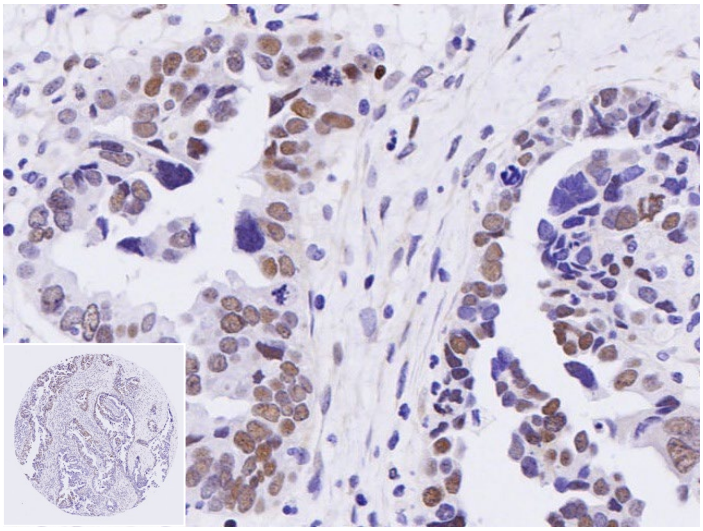
Enhanced validation data

c-Myc

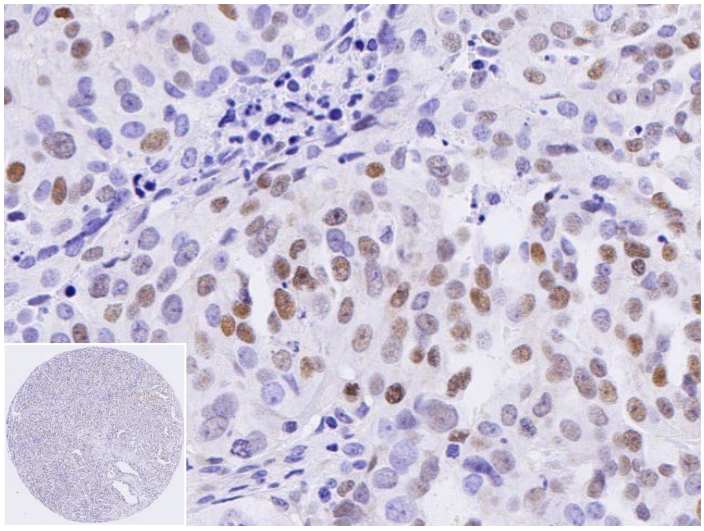
Serous cystadenocarcinoma (48.46)



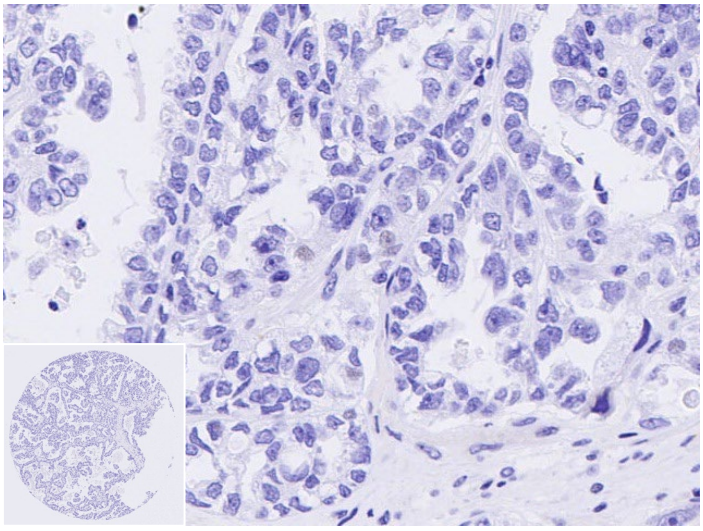
Serous cystadenocarcinoma (26.21)



Serous cystadenocarcinoma (16.49)



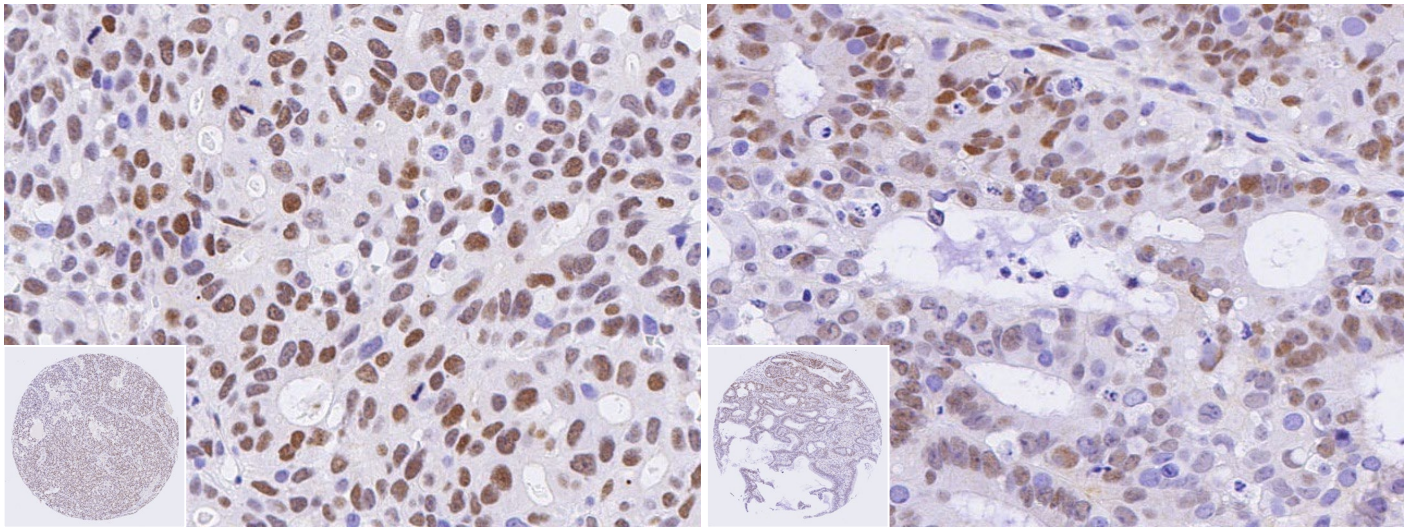
Serous cystadenocarcinoma (0.06)



c-Myc

Endometrioid adenocarcinoma (85.30)

Endometrioid adenocarcinoma (45.31)



Mucinous cystadenocarcinoma (72.32)

Mucinous cystadenocarcinoma (40.35)

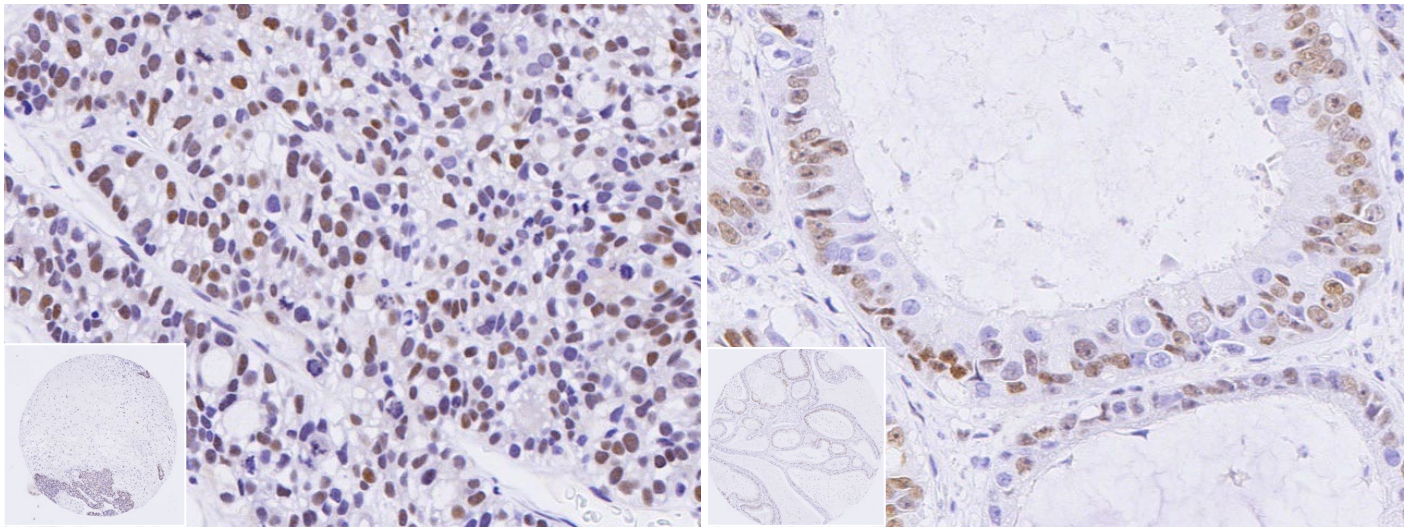


Figure 7. c-Myc expression in ovarian cancer IHC images show weak, moderate or strong staining in brown (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

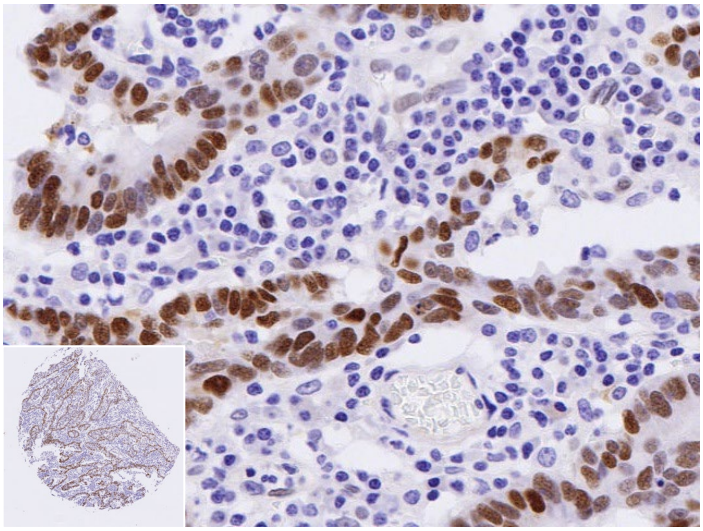
NanoZoomer® is a registered trademark of Hamamatsu Photonics K.K.

c-Myc expression in stomach cancer TMA (BOND™ RX)

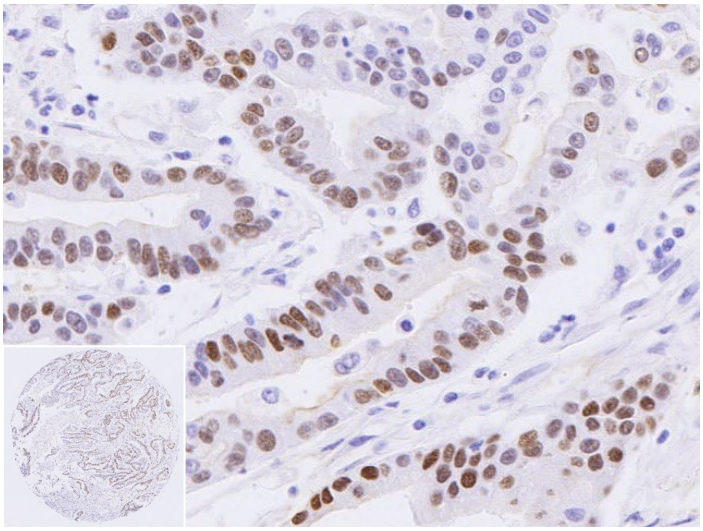
Below are the representative images of human stomach cancer TMA showing strong to weak c-Myc expression.

c-Myc

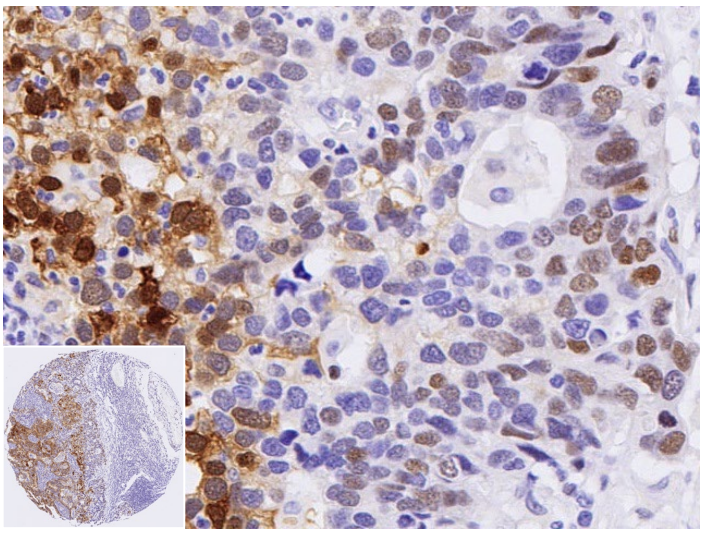
Stomach adenocarcinoma (93.72)



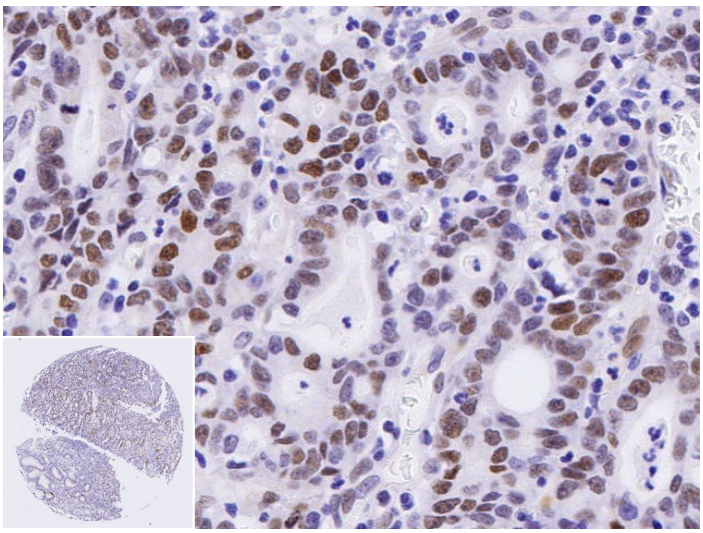
Stomach adenocarcinoma (90.27)



Stomach adenocarcinoma (85.34)



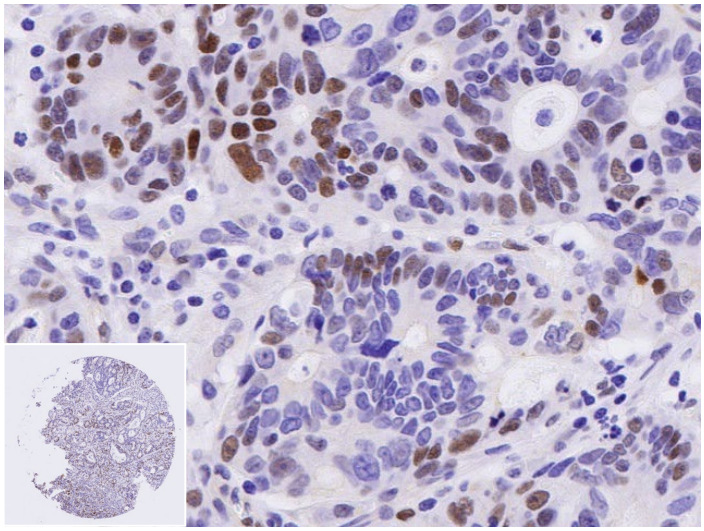
Stomach adenocarcinoma (50.82)



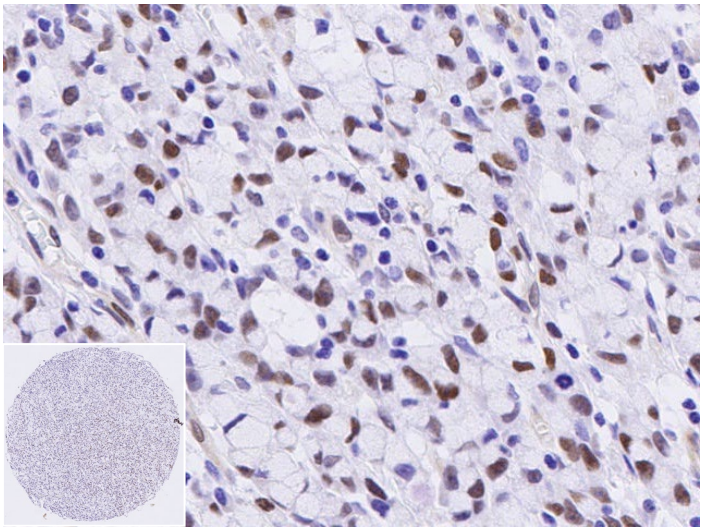
Enhanced validation data

c-Myc

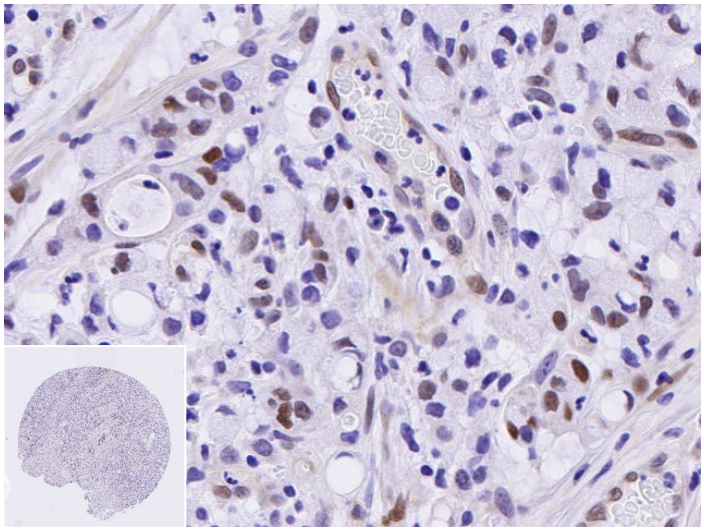
Stomach adenocarcinoma (44.84)



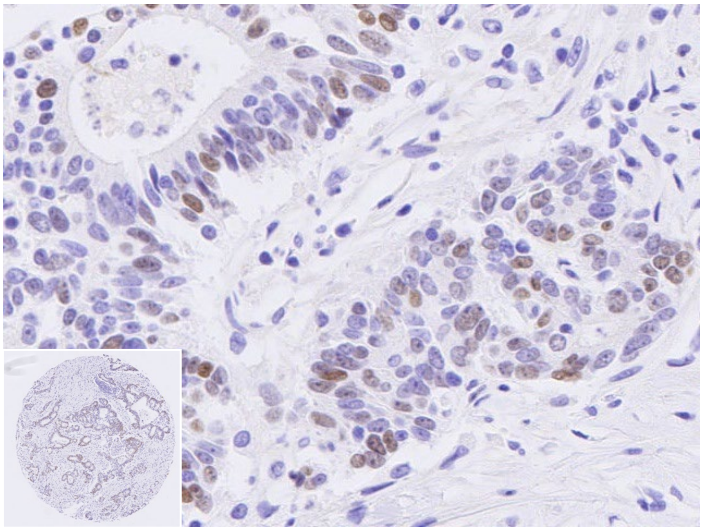
Stomach adenocarcinoma (34.10)



Stomach adenocarcinoma (32.48)



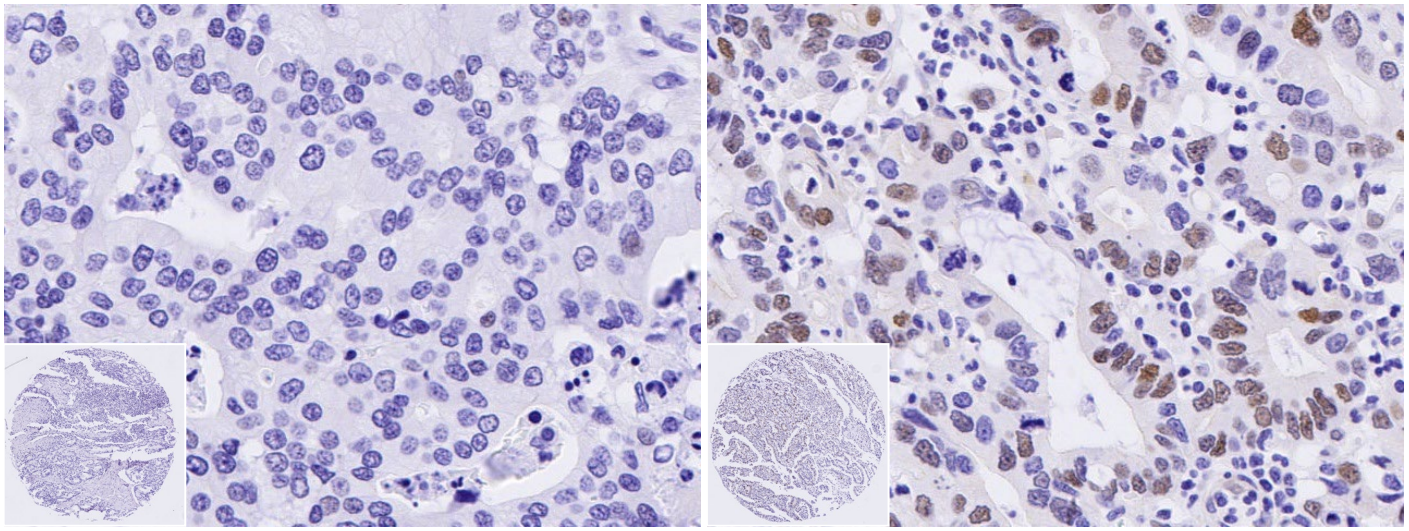
Stomach adenocarcinoma (26.52)



c-Myc

Stomach adenocarcinoma (0.16)

Mucinous adenocarcinoma (16.64)



Undifferentiated carcinoma (0.19)

Undifferentiated carcinoma (0.11)

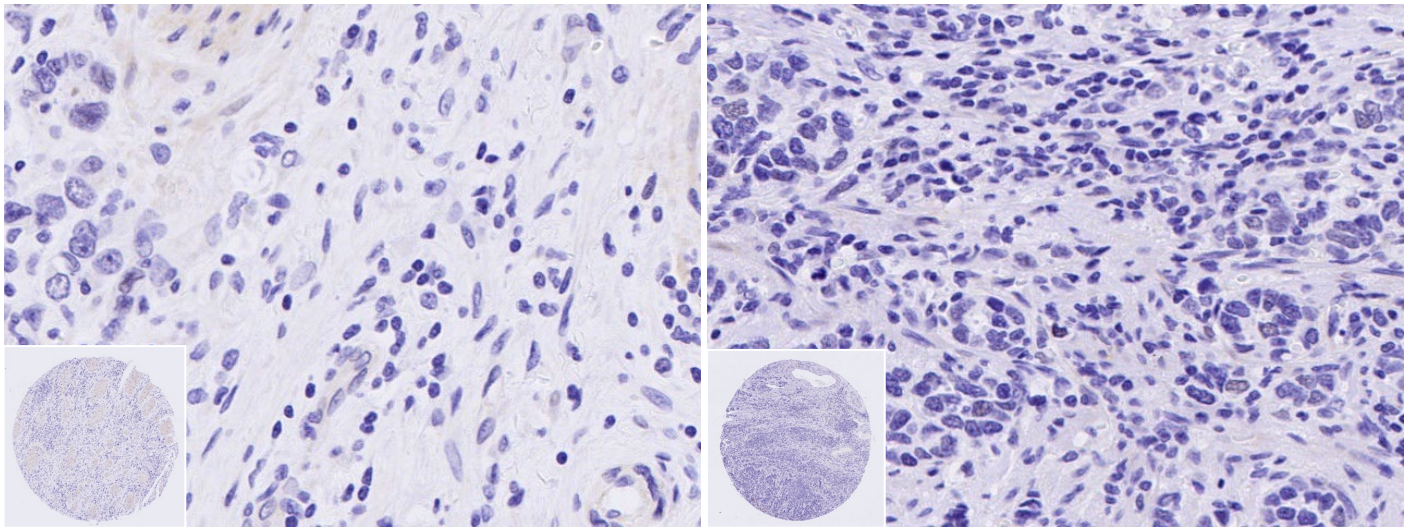


Figure 8. c-Myc expression in stomach cancer. IHC images show weak, moderate or strong staining in brown (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

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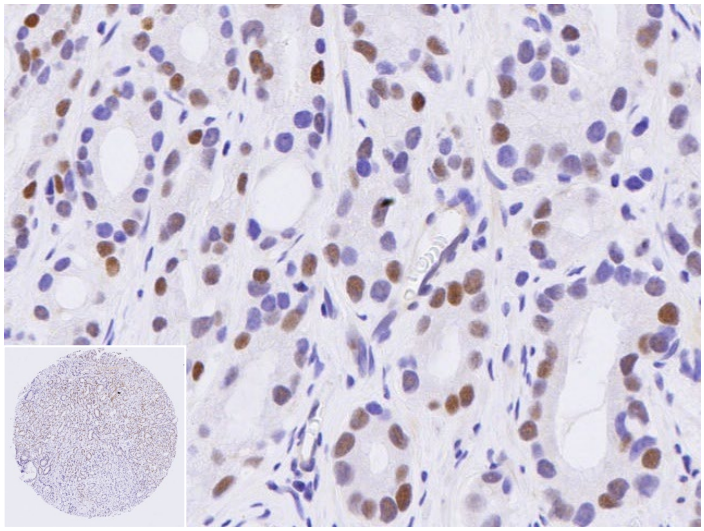
c-Myc expression in prostate cancer TMA (BOND™ RX)

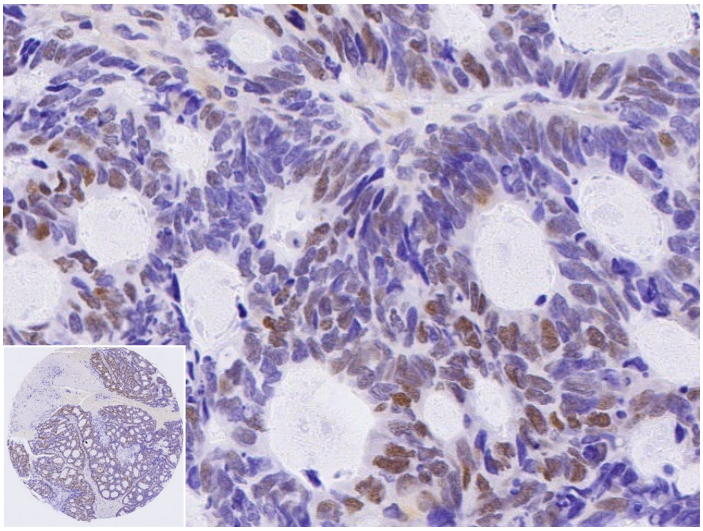
Below are the representative images of human prostate cancer TMA showing strong to weak c-Myc expression.

c-Myc

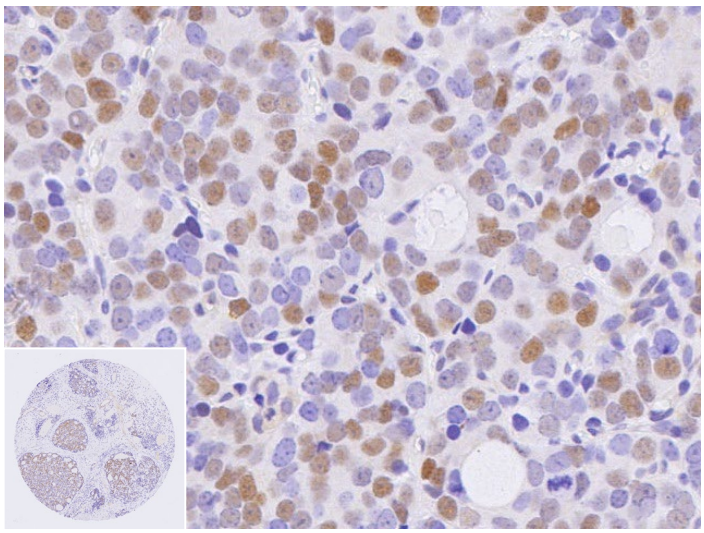
Prostate adenocarcinoma (68.74)



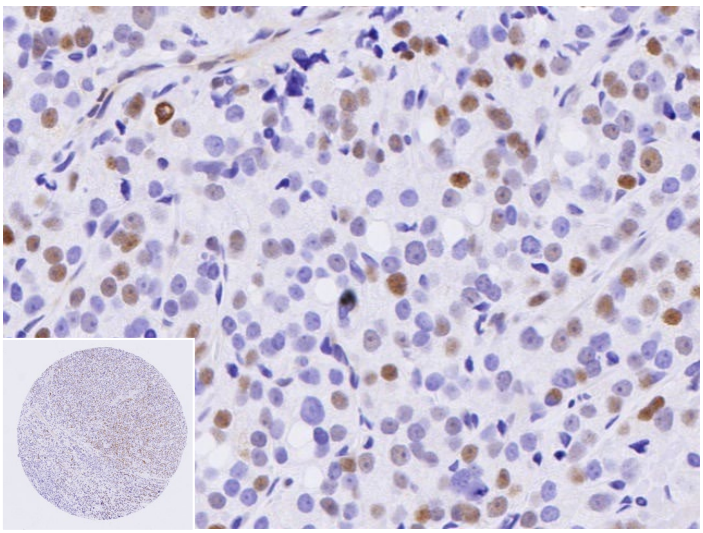
Prostate adenocarcinoma (49.87)



Prostate adenocarcinoma (36.81)



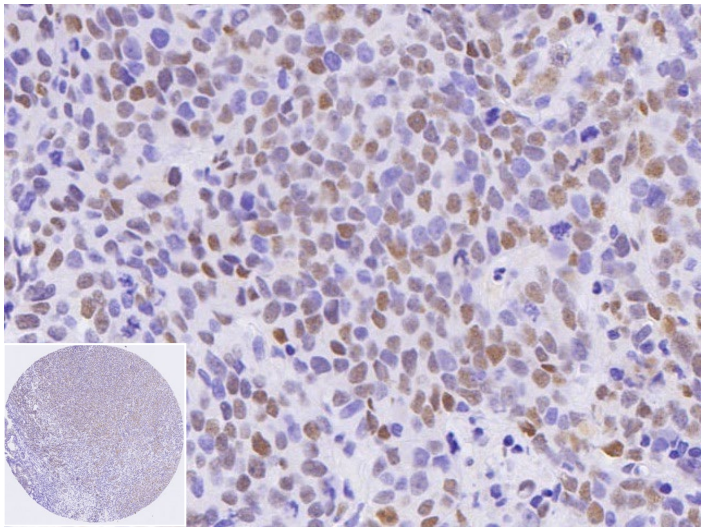
Prostate adenocarcinoma (36.44)



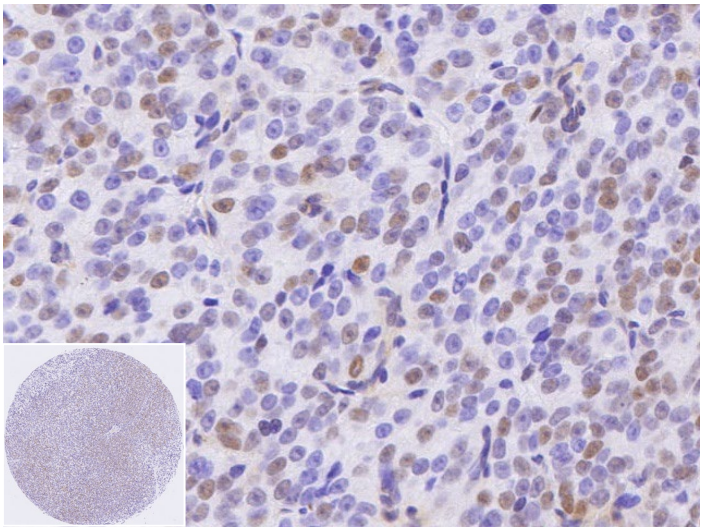
Enhanced validation data

c-Myc

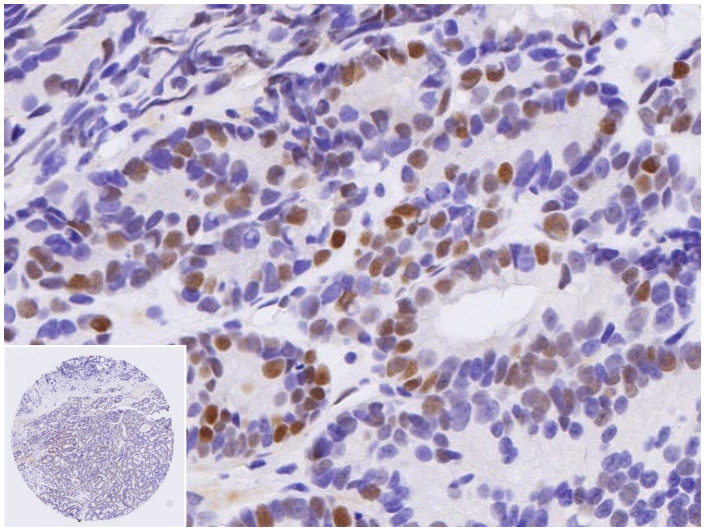
Prostate adenocarcinoma (30.60)



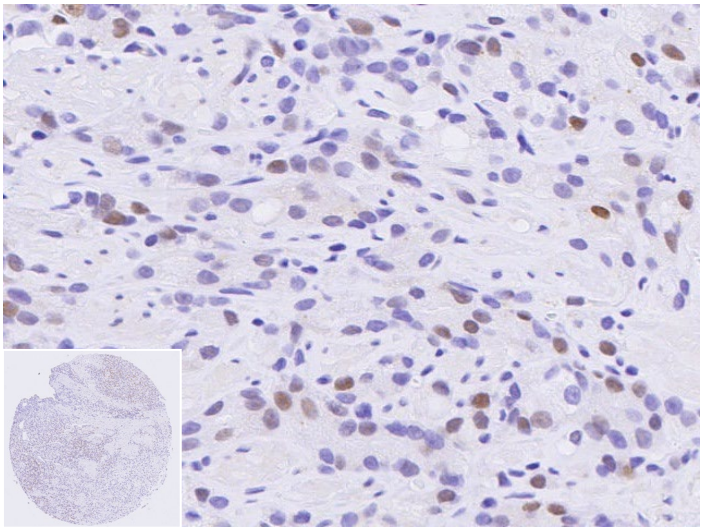
Prostate adenocarcinoma (24.40)



Prostate adenocarcinoma (22.79)

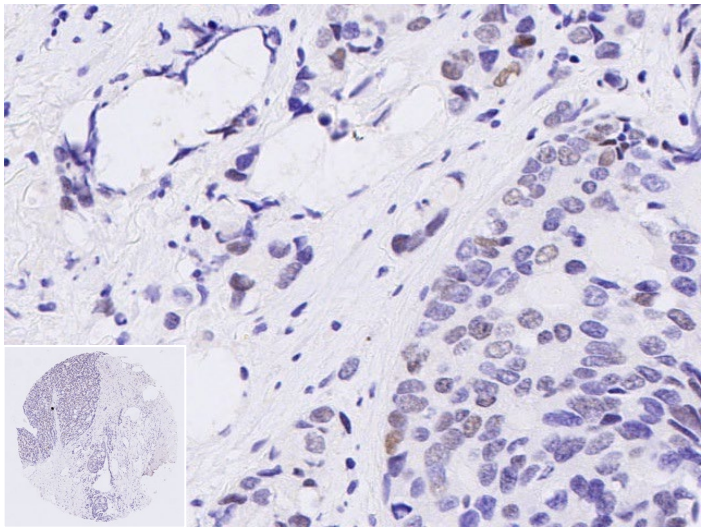


Prostate adenocarcinoma (12.83)

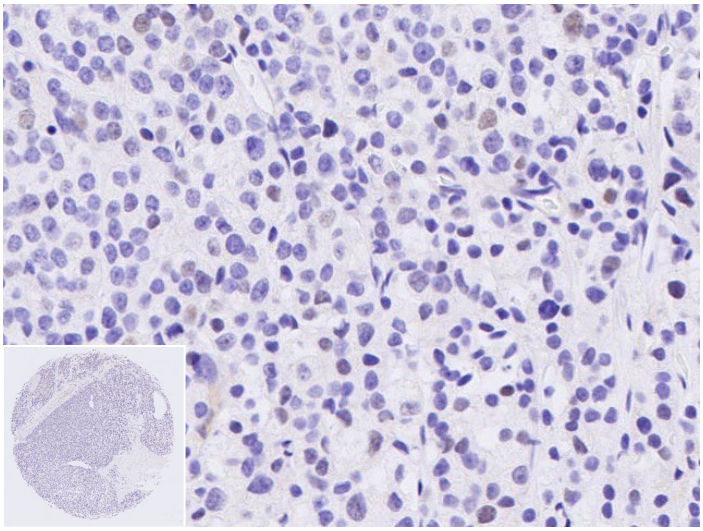


c-Myc

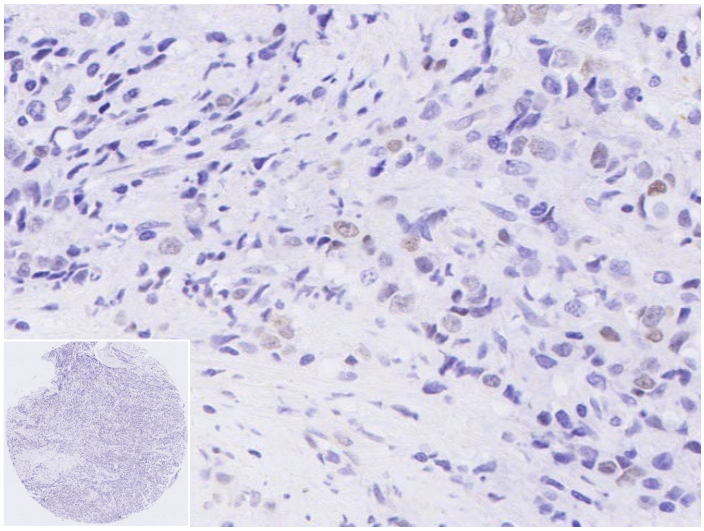
Prostate adenocarcinoma (12.45)



Prostate adenocarcinoma (3.28)



Prostate adenocarcinoma (1.04)



Prostate adenocarcinoma (0.02)

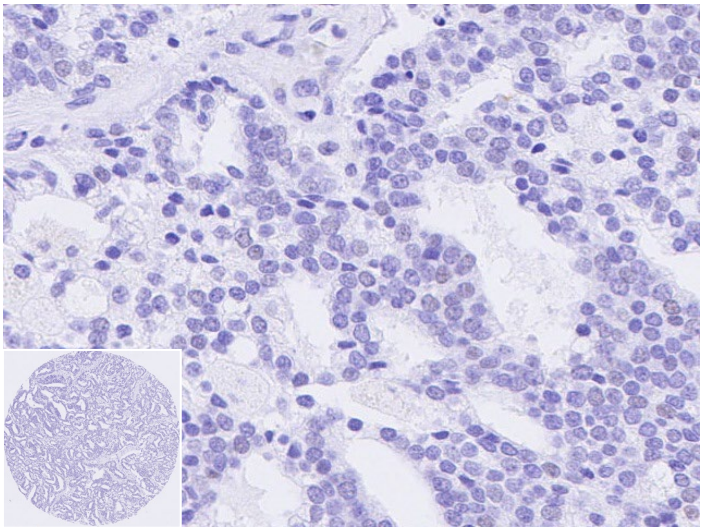


Figure 9. c-Myc expression in prostate cancer. IHC images show weak, moderate or strong staining in brown (a-l); nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZommer S360 (Hamamatsu Photonics K.K.) and and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope

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