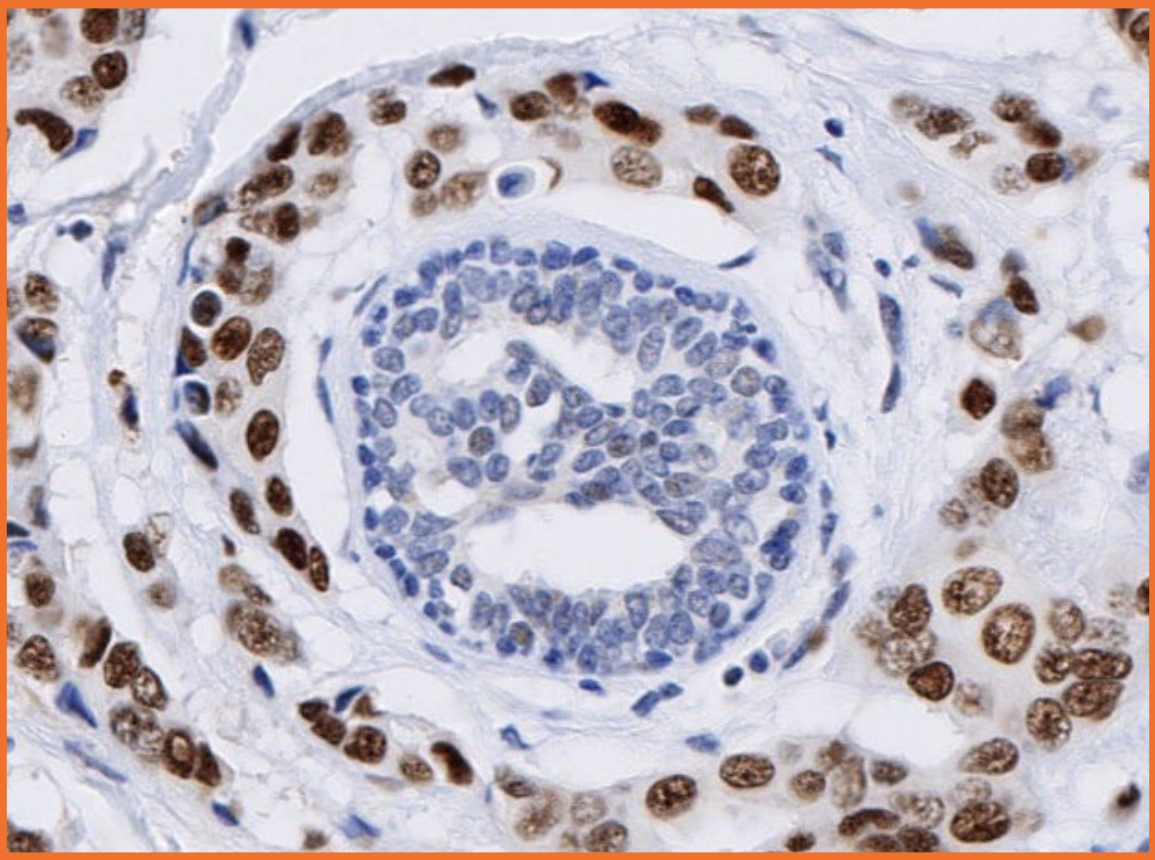
Enhanced validation data

Anti-CDK12 recombinant antibody – ab317746



CDK12 expression in breast carcinoma

progress happens together

abcam

AB0455_v1.0_111025

Enhanced validation of Anti-CDK12 recombinant antibody [EPR29009-30] – ab317746

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 tissue microarrays (TMAs).

— In this study, we demonstrate the sensitivity and specificity of Anti-CDK12 antibody (ab317746) in IHC in selected tissues and TMAs using a BOND™ RX Research Stainer (Leica®) and DISCOVERY ULTRA system (Roche Diagnostics).

Target overview

HGNC symbol
CDK12

Approved name
Cyclin dependent kinase 12

Chromosomal location
17q12

Function

CDK12 has several important functions in cellular processes: transcription regulation[1], DNA damage response[2], mRNA processing[3], cell cycle progression[4] and tumor suppression and oncogenesis[5].

Tissue specificity

CDK12 is expressed in various normal tissues, including the brain, testis, and immune cells. High expression levels have been observed in several cancers, such as breast, ovarian, prostate, and colorectal cancers[6].

Cellular localization

Nucleus

Database links

Entrez Gene: 51755
OMIM®: 615514
Uniprot: Q9NYV4

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[7-10].

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

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 CC1)	32 min, 100 °C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Anti-CDK12 antibody [EPR29009-30] -ab317746 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).

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-CDK12 antibody [EPR29009-30] -ab317746 diluted in Bond™ primary antibody diluent (#AR9352) to final concentration of 0.1 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
Wash	Deionized water	1x	0
	Bond™ wash solution	1x	0
	Deionized water	1x	0

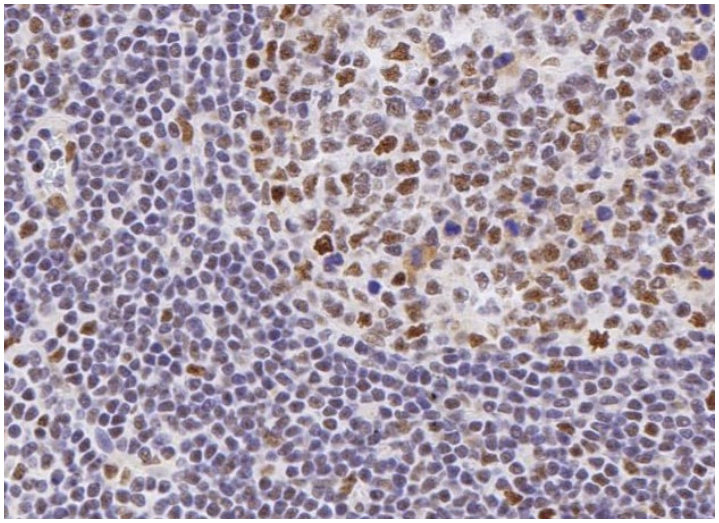
Table 3. IHC staining protocol on BOND™ RX Research Stainer (Leica®). The protocol used is the same as the default IHC protocol F on BOND™ RX Research Stainer (Leica®), apart from the standard post-primary step, which has been excluded from our protocol. All steps were performed at room temperature.

CDK12 expression in multi-normal TMA (BOND™ RX)

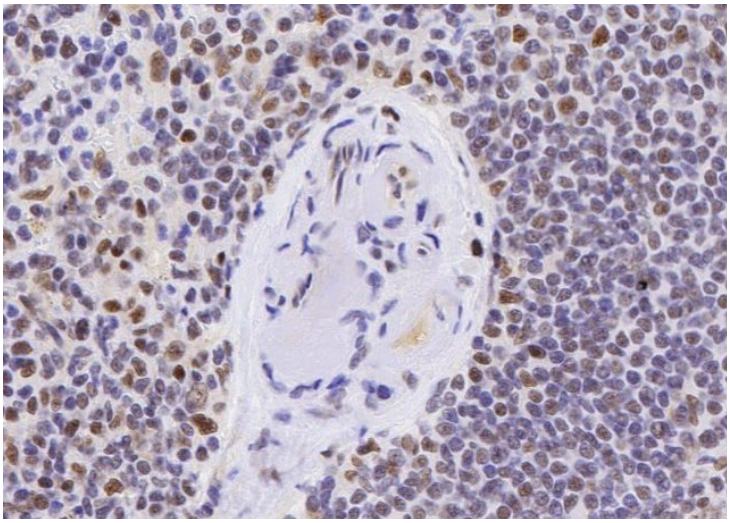
Below are the representative images of selected tissues from multi-normal TMA. CDK12 expression was detected in the tonsil, spleen, lung, testis, skin colon and pancreas whereas low to no expression was observed in liver, brain, prostate, and heart tissues.

CDK12

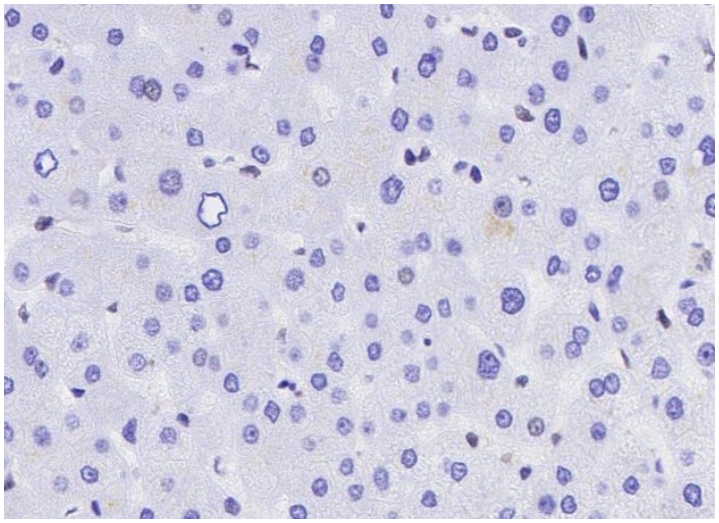
Tonsil



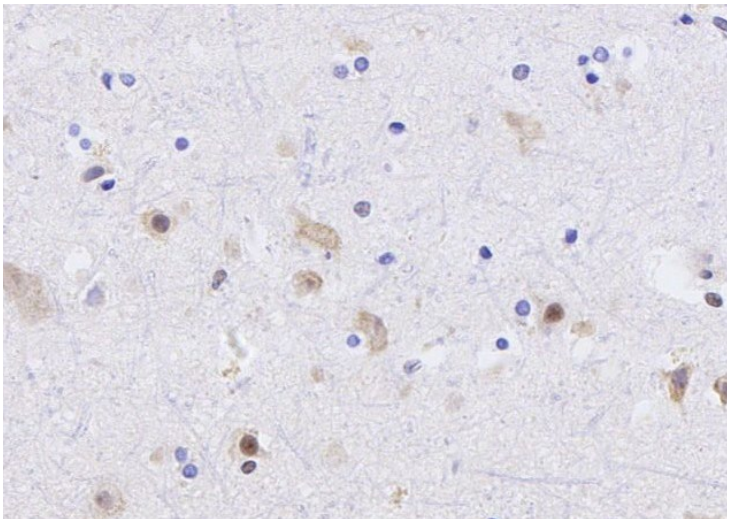
Spleen



Liver

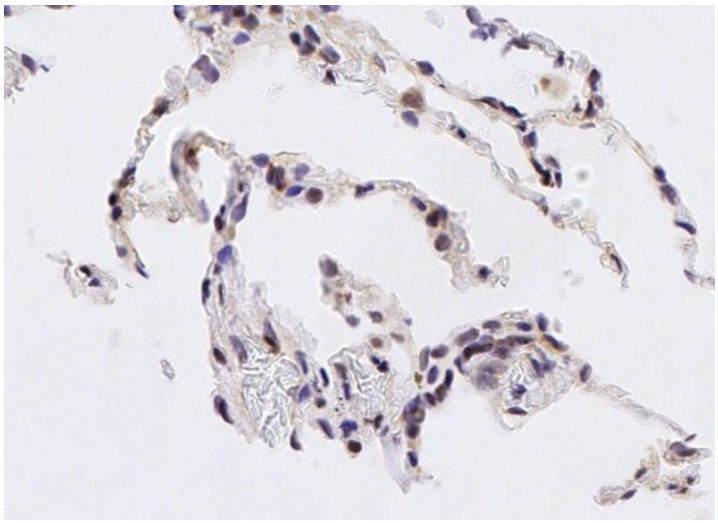


Brain (cerebrum)

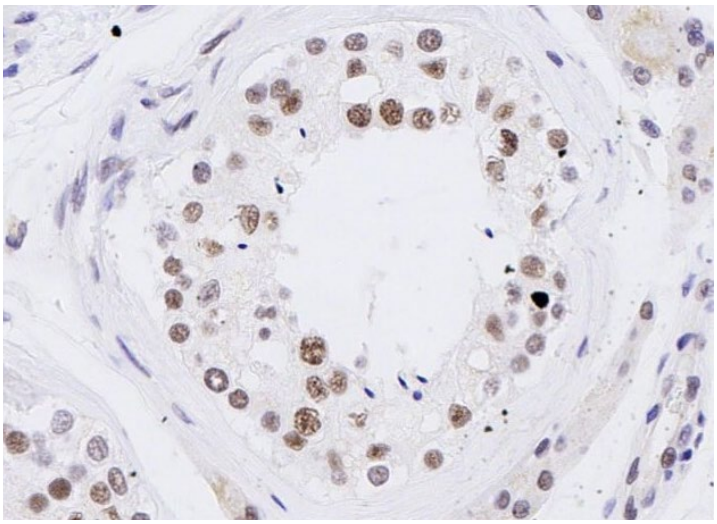


Enhanced validation data

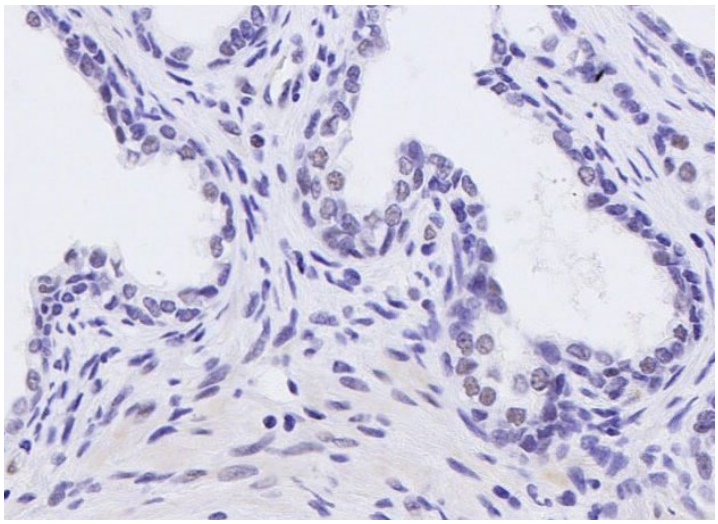
Lung



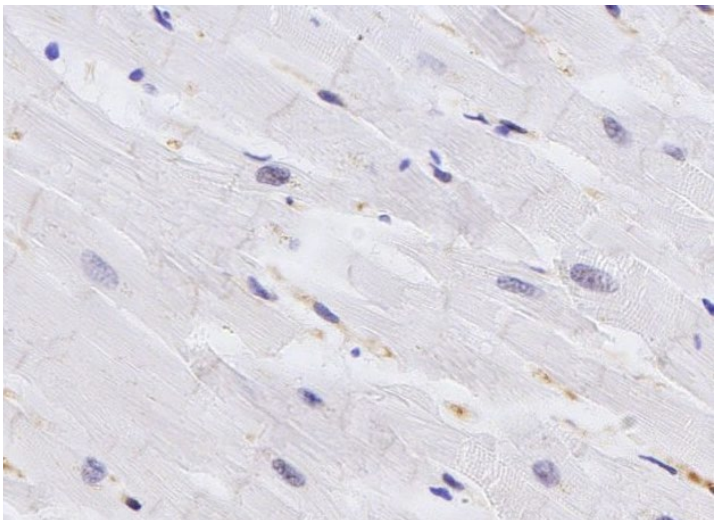
Testis



Prostate

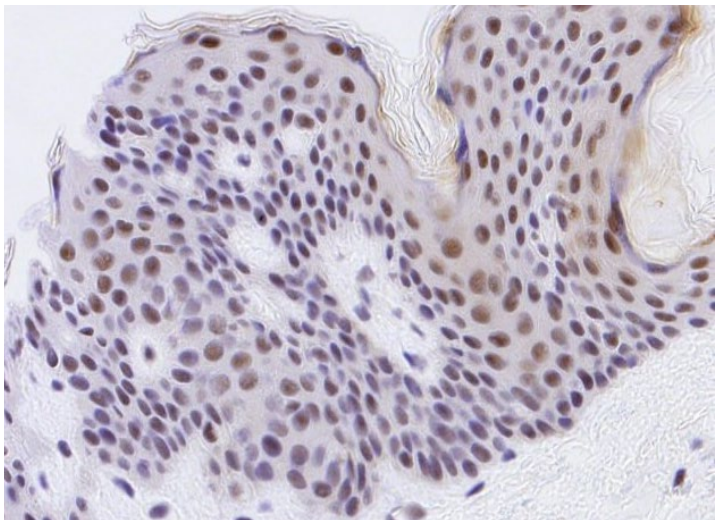


Heart

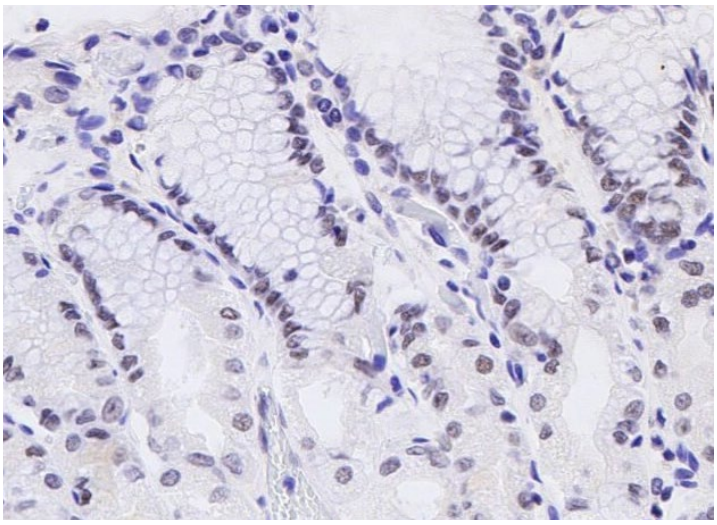


Enhanced validation data

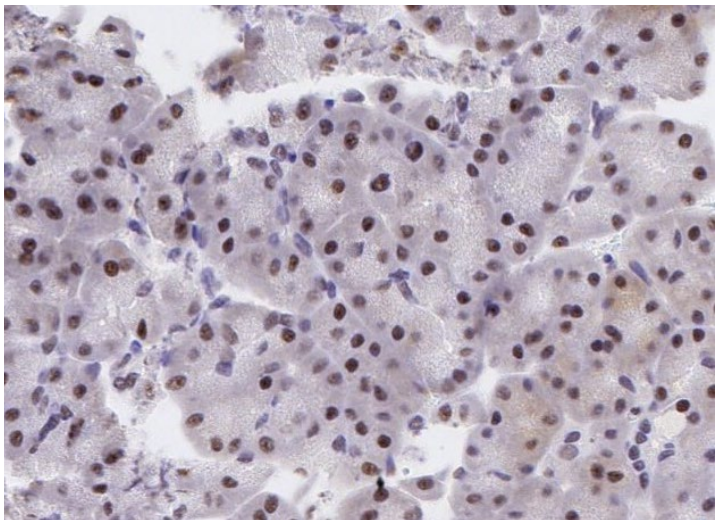
Skin



Stomach



Pancreas



Pancreas - Isotype control

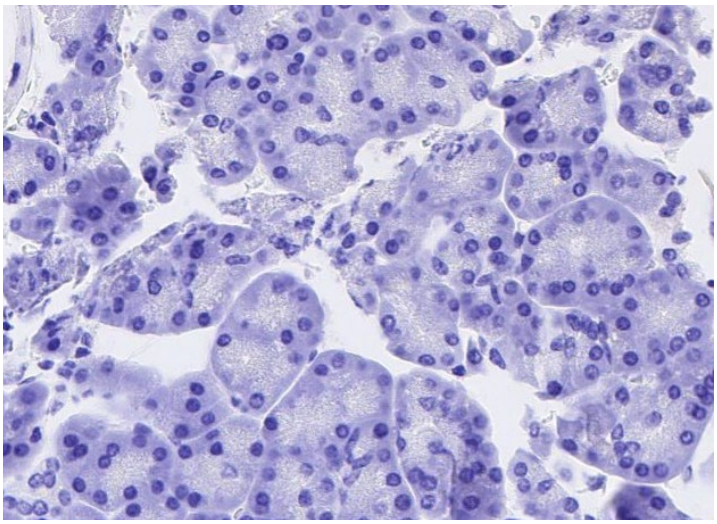


Figure 1. CDK12 expression in human normal tissue. IHC staining of multi-normal human tissues using anti-CDK12 (ab317746) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue.
Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

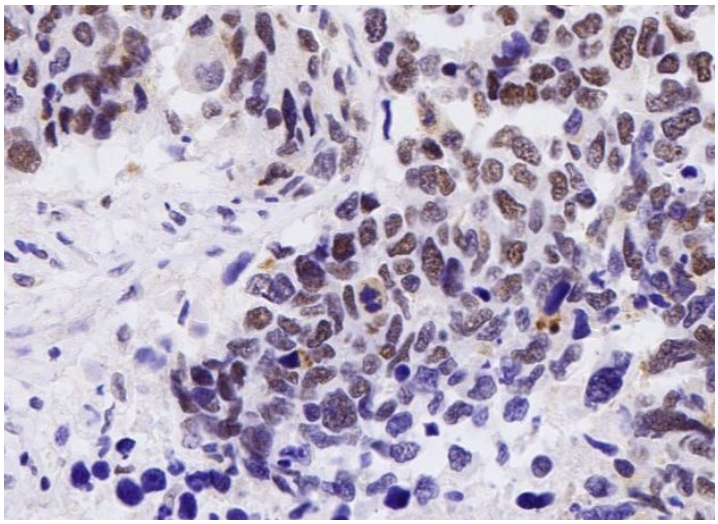
NanoZoomer® is a registered trademark of Hamamatsu Photonics K.K.

CDK12 expression in multi-cancer TMA (BOND™ RX)

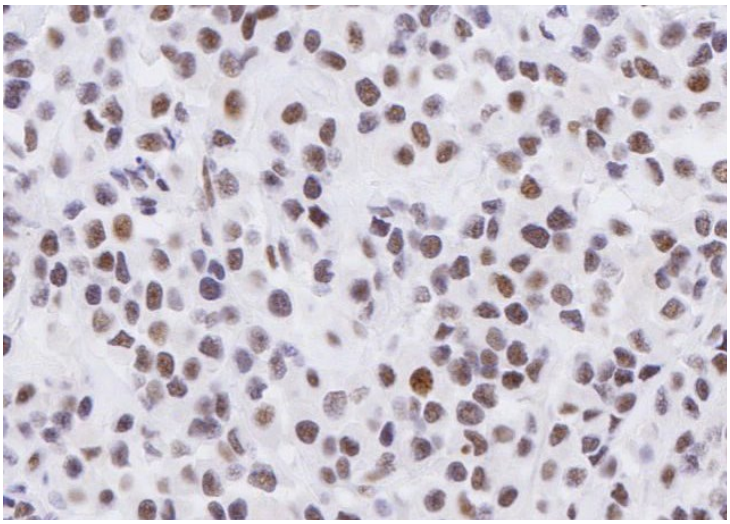
Below are the representative images of selected tissues from multi-cancer TMA. CDK12 expression was detected in breast cancer (ductal and invasive lobular carcinoma), glioblastoma, non-small cell lung carcinoma, adenocarcinoma of the stomach, colon, pancreas, and prostate; endometrial cancer, bladder carcinoma, renal cell carcinoma.

CDK12

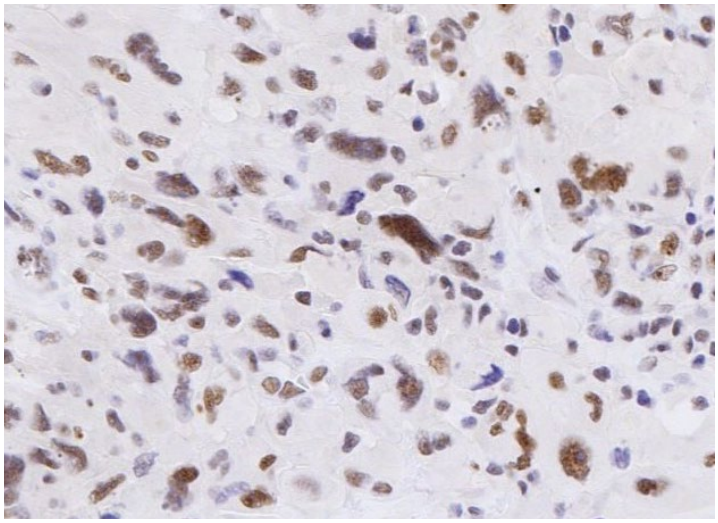
Breast ductal carcinoma



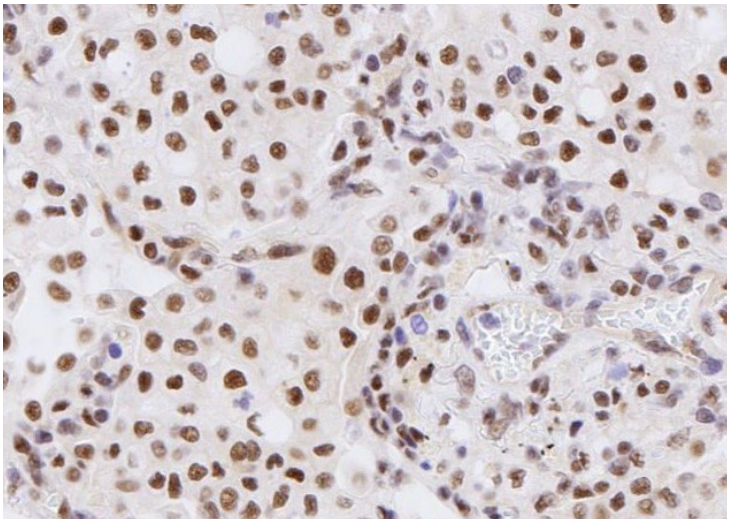
Breast invasive lobular carcinoma



Glioblastoma

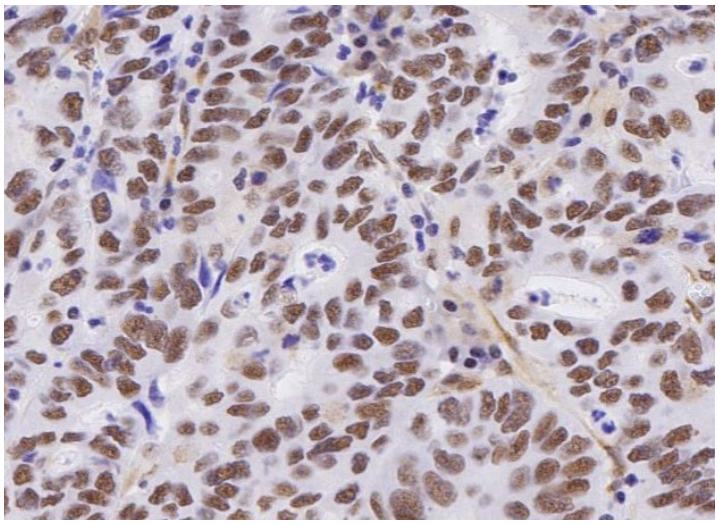


Non-small cell lung cancer

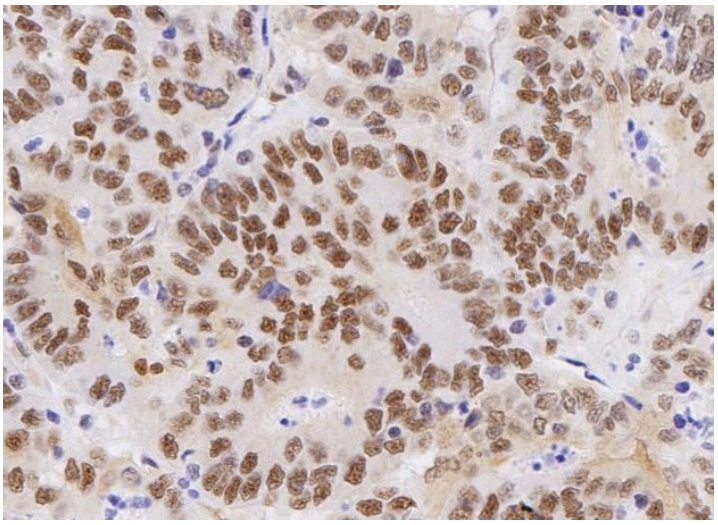


Enhanced validation data

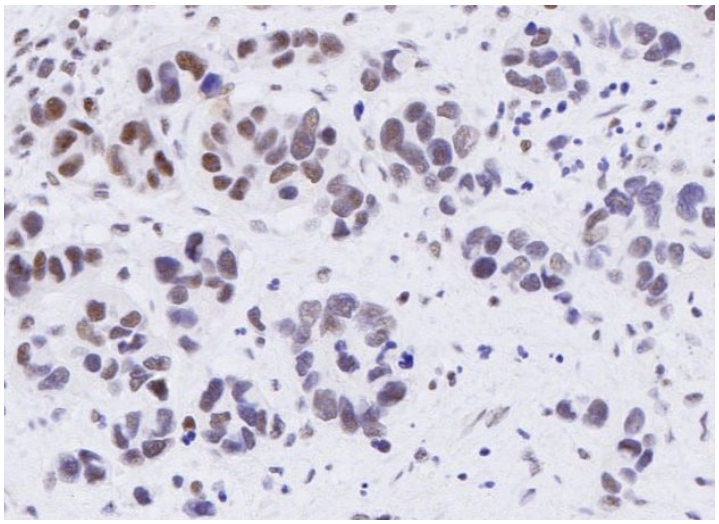
Stomach adenocarcinoma



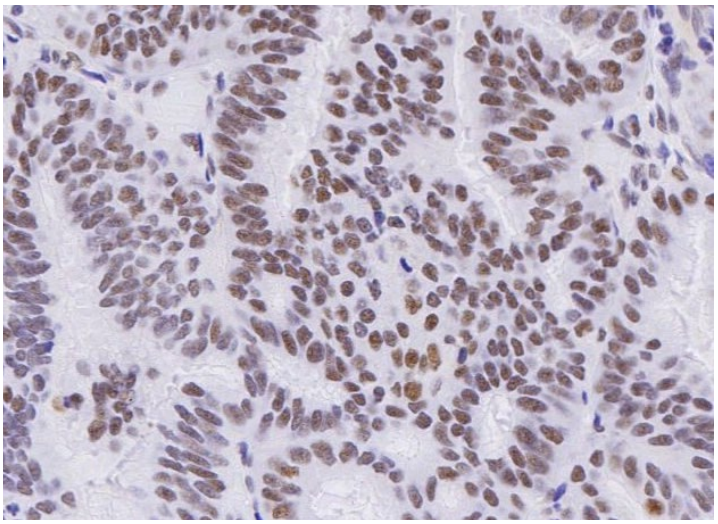
Colon adenocarcinoma



Pancreatic adenocarcinoma

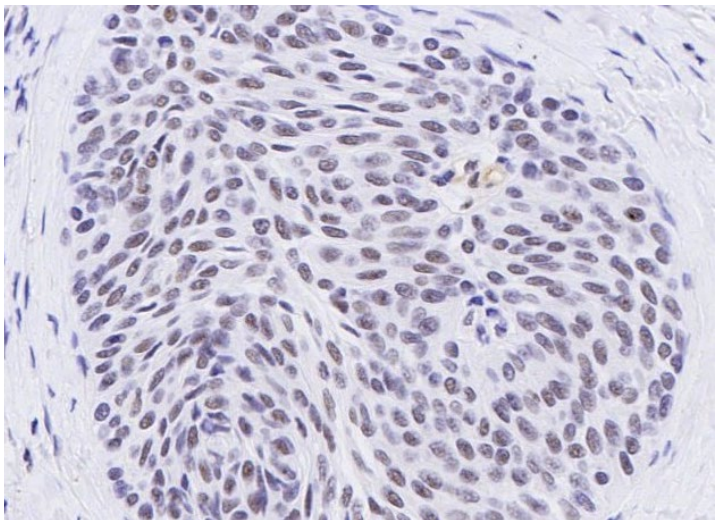


Endometrial cancer

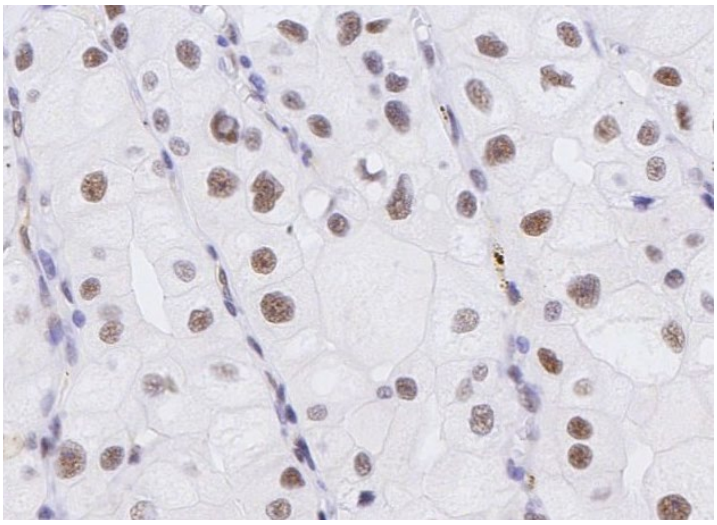


Enhanced validation data

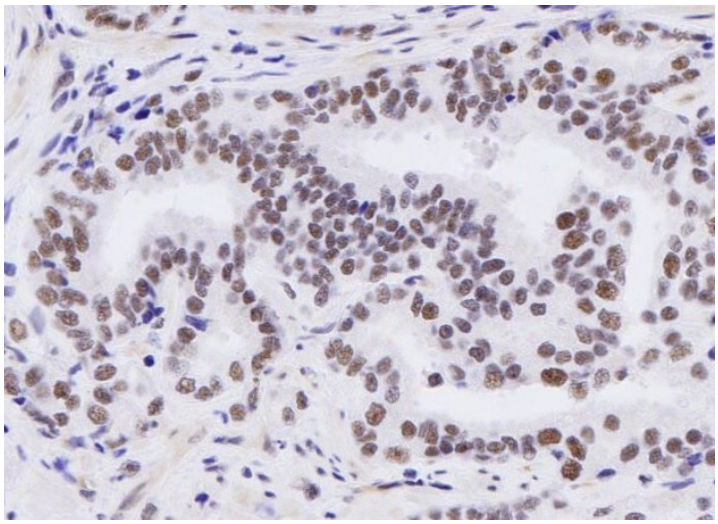
Bladder carcinoma



Renal cell carcinoma



Prostate adenocarcinoma



Prostate adenocarcinoma - Isotype control

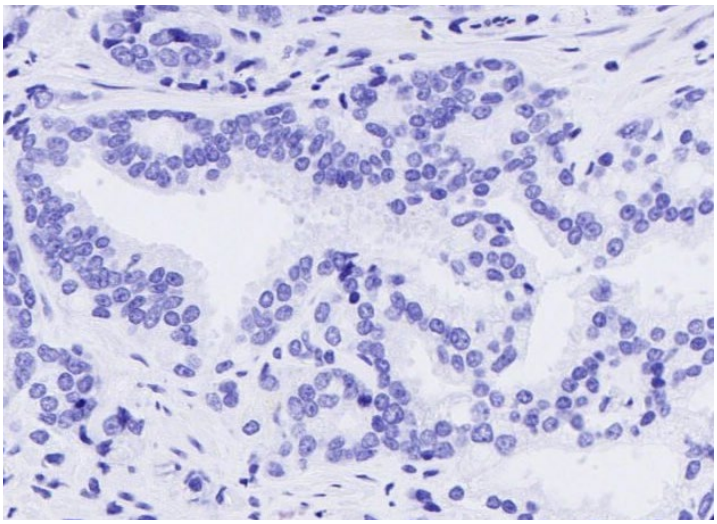


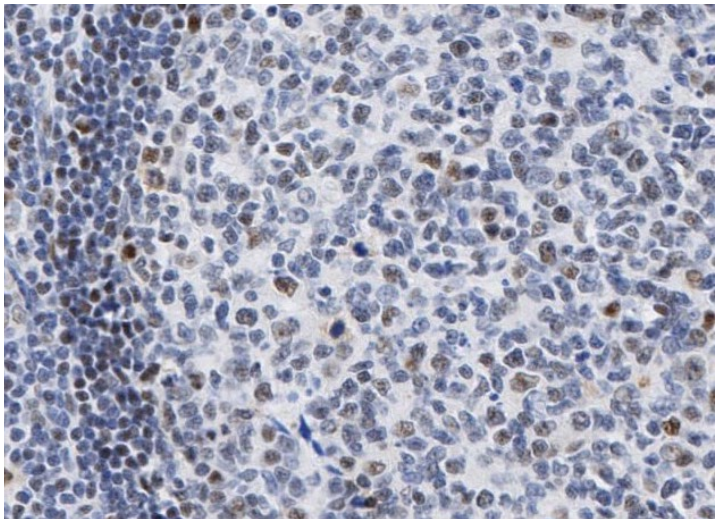
Figure 2. CDK12 expression in cancer. IHC staining of multi-cancer human tissues using anti-CDK12 (ab317746) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

CDK12 expression in multi-normal TMA (DISCOVERY ULTRA)

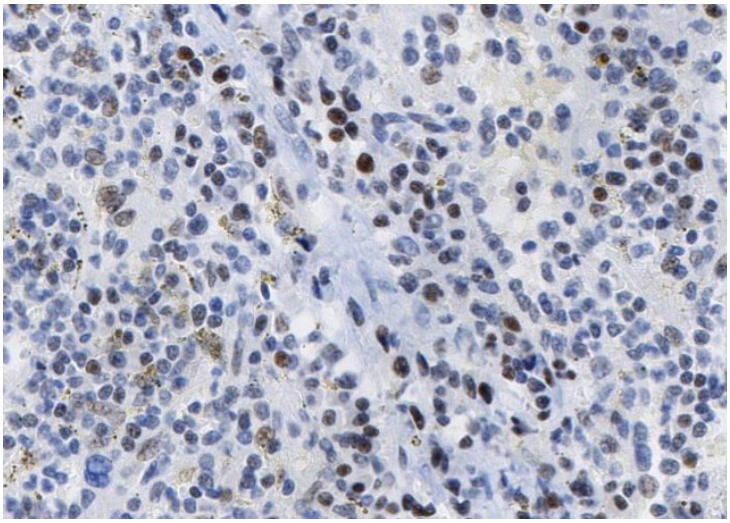
Below are the representative images of selected tissues from multi-normal TMA. CDK12 expression was detected in the tonsil, spleen, lung, testis, skin colon and pancreas whereas low to no expression was observed in liver, brain, prostate, and heart tissues.

CDK12

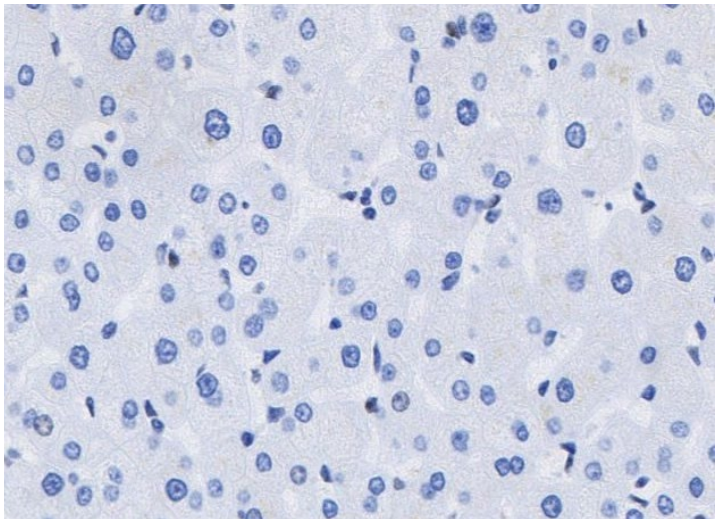
Tonsil



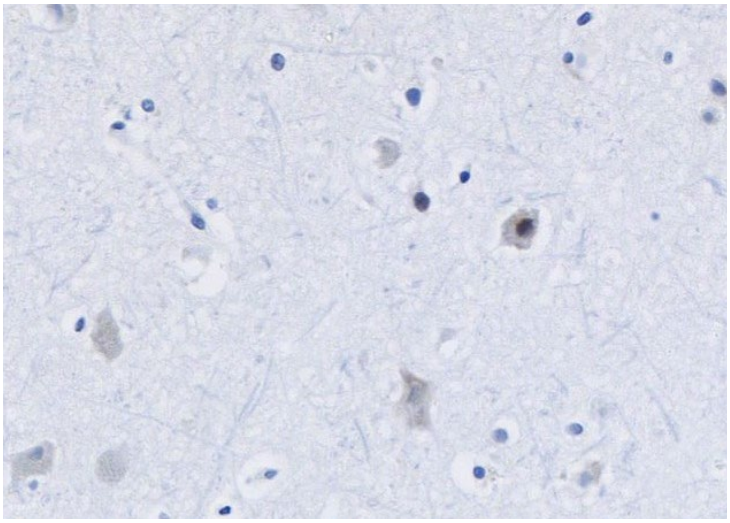
Spleen



Liver

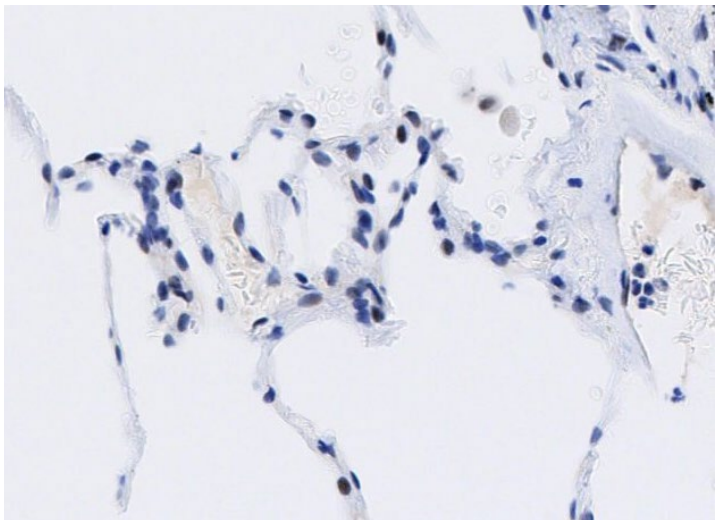


Brain (cerebrum)

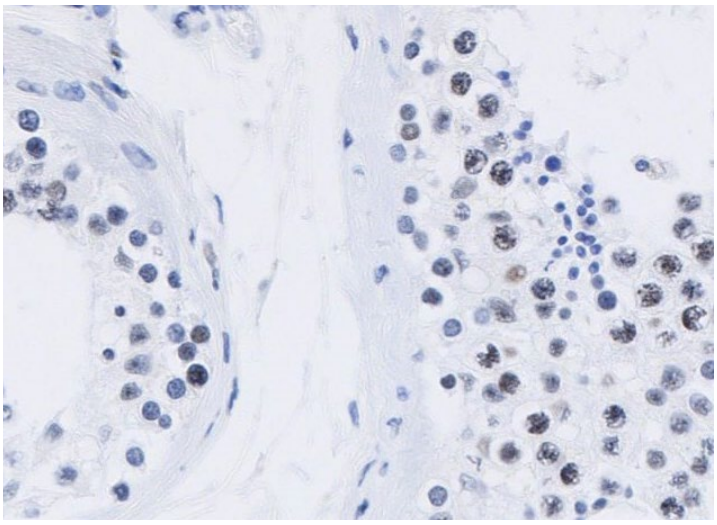


Enhanced validation data

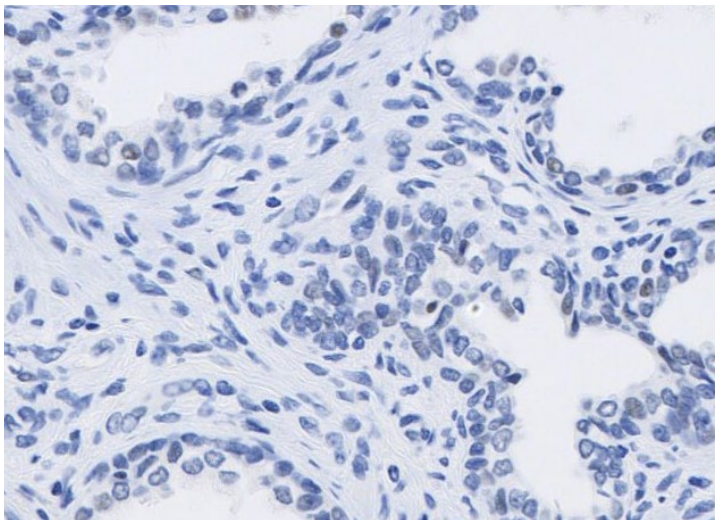
Lung



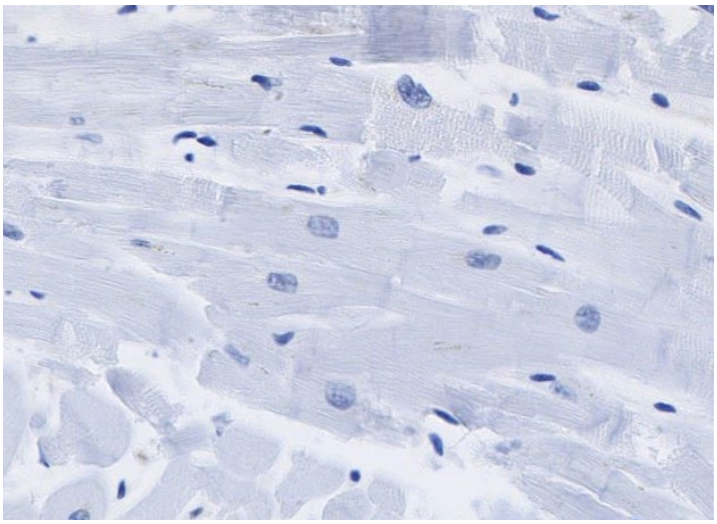
Testis



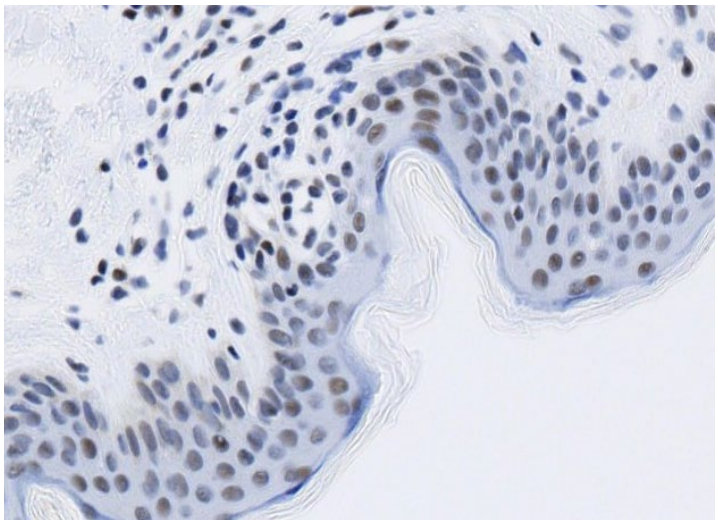
Prostate



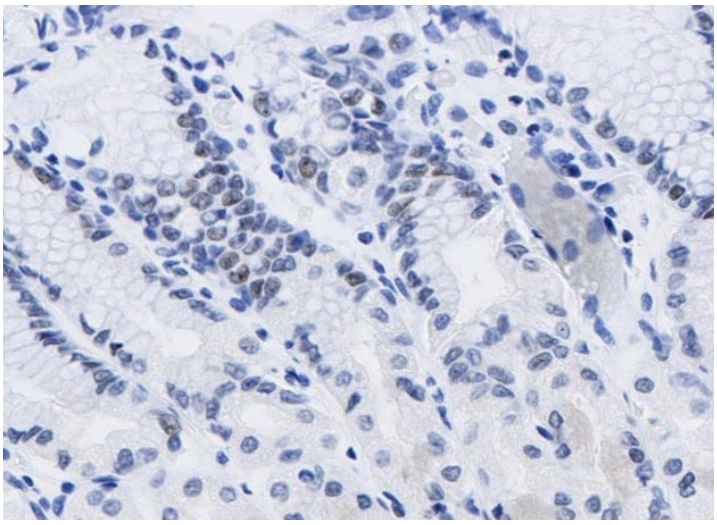
Heart



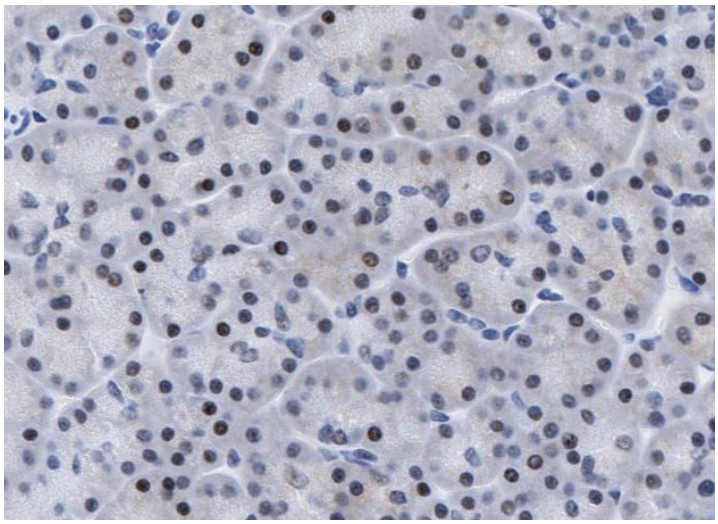
Skin



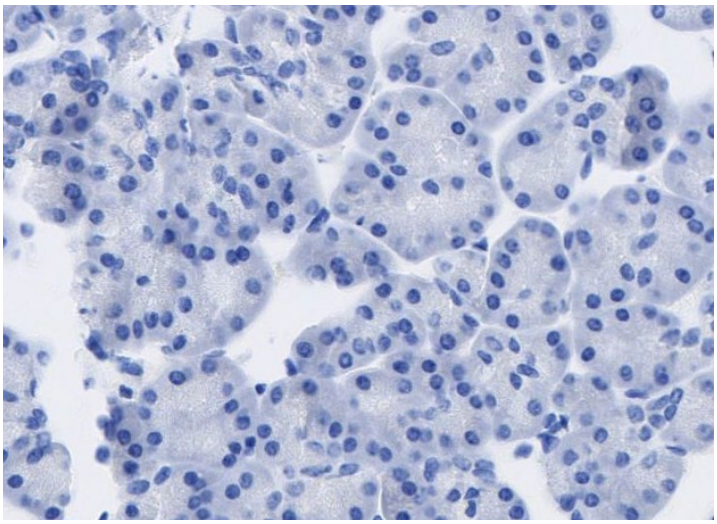
Stomach



Pancreas



Pancreas - Isotype control



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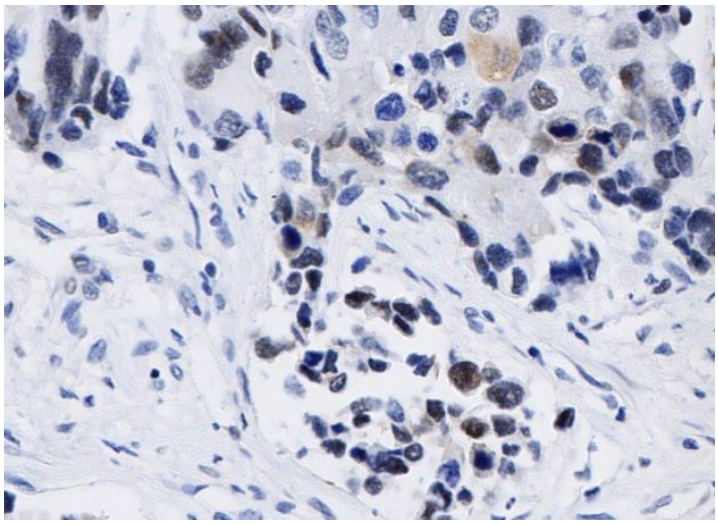
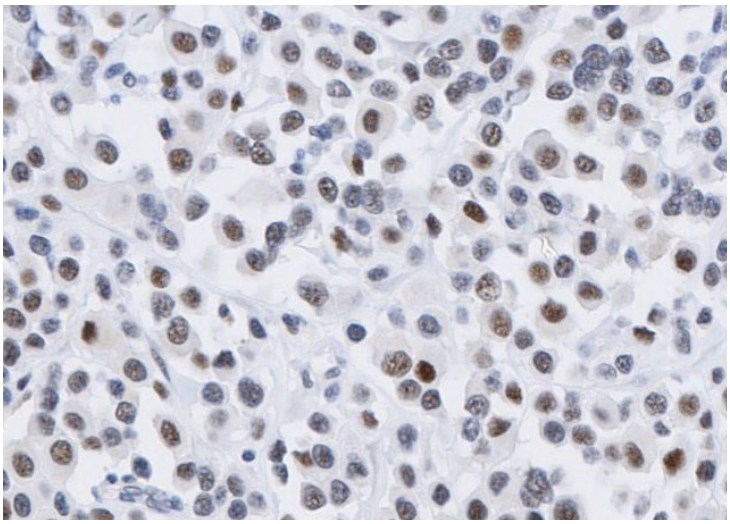
CDK12

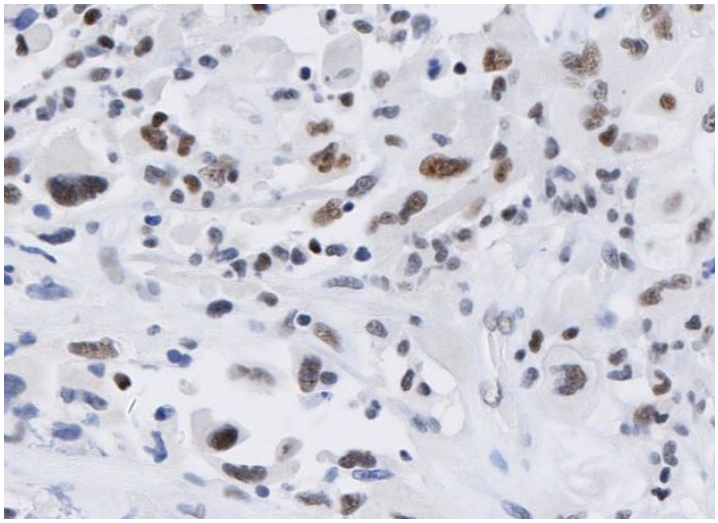
Breast ductal carcinoma



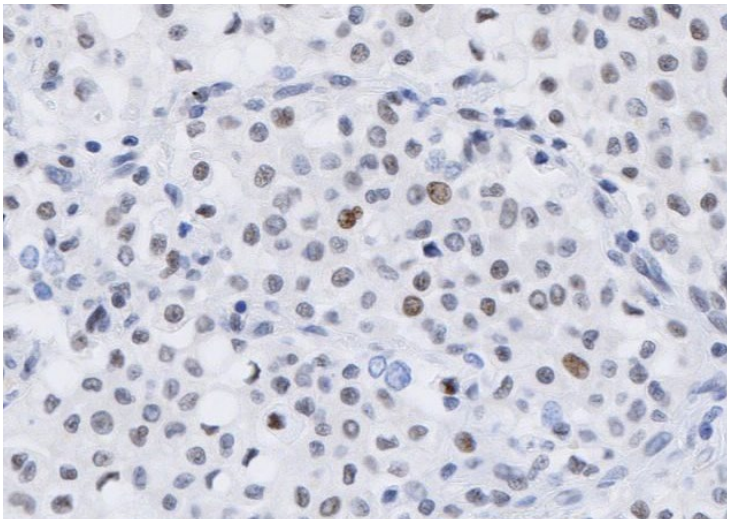
Breast invasive lobular carcinoma



Glioblastoma

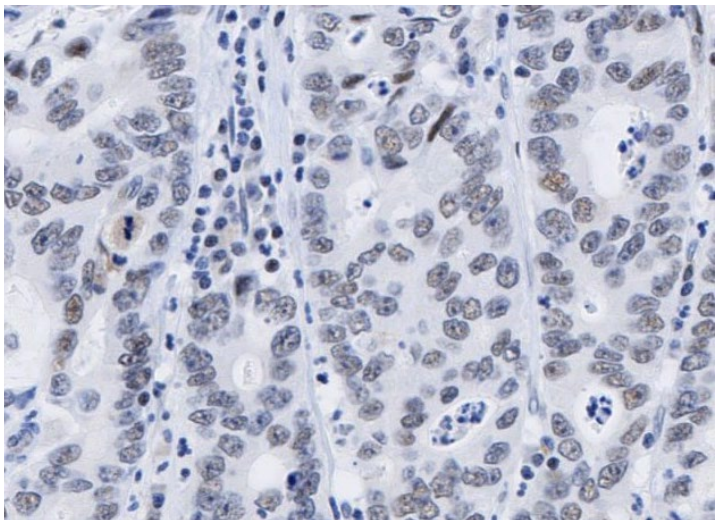


Non-small cell lung cancer

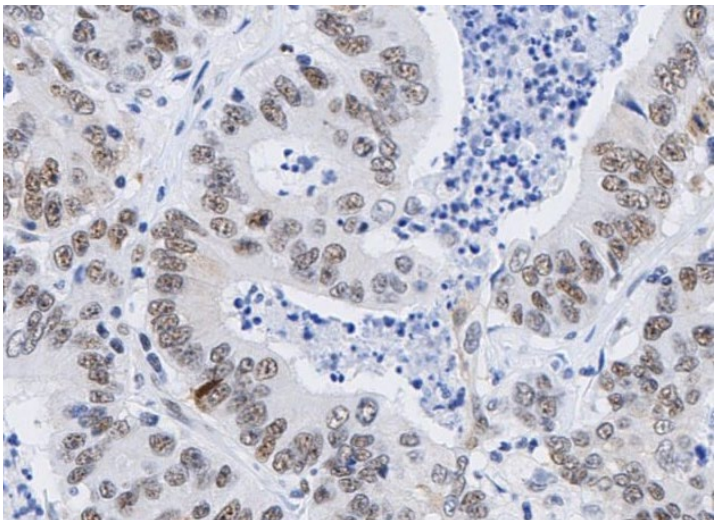


Enhanced validation data

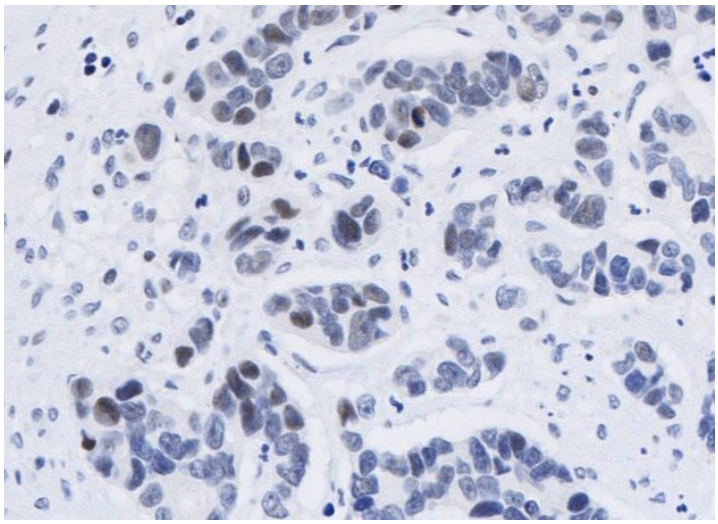
Stomach adenocarcinoma



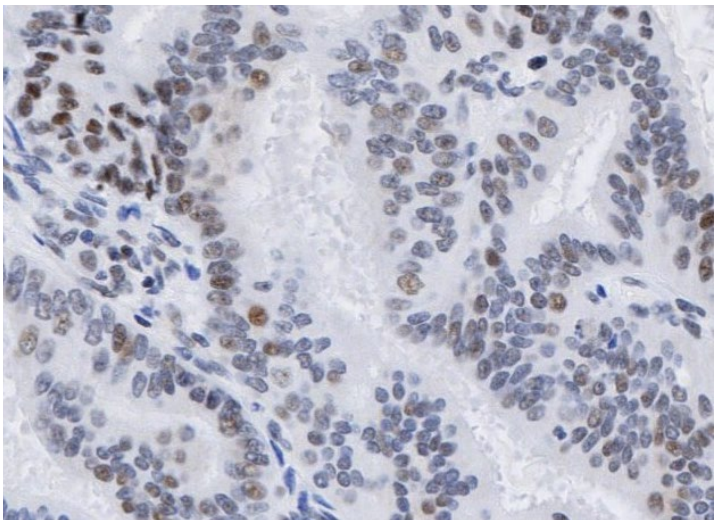
Colon adenocarcinoma



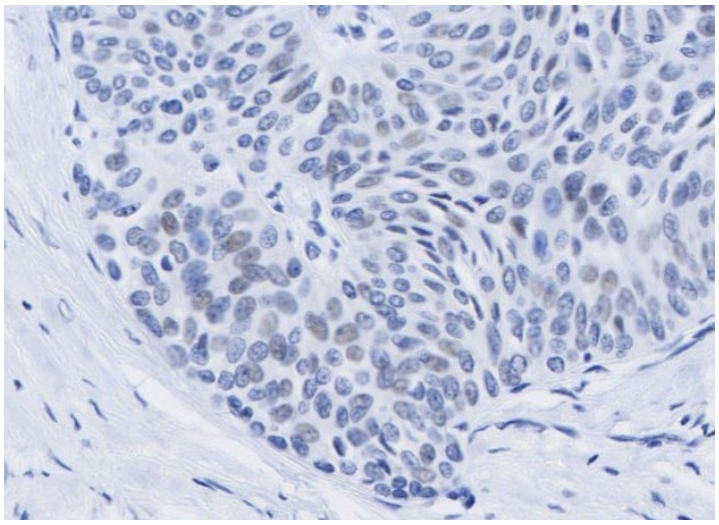
Pancreatic adenocarcinoma



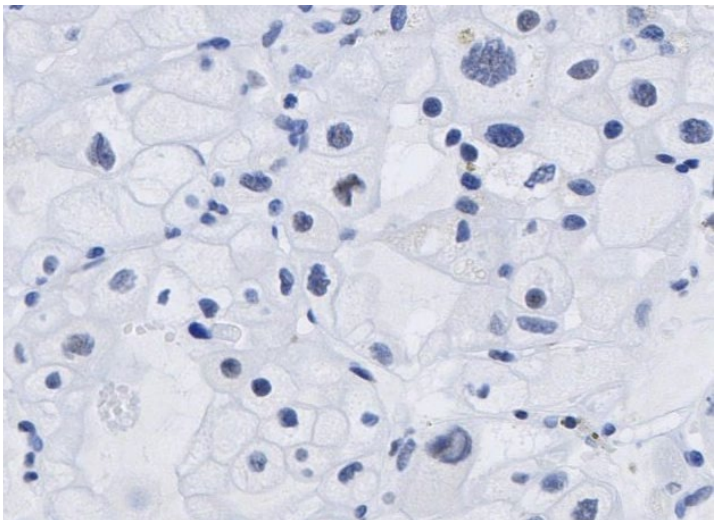
Endometrial cancer



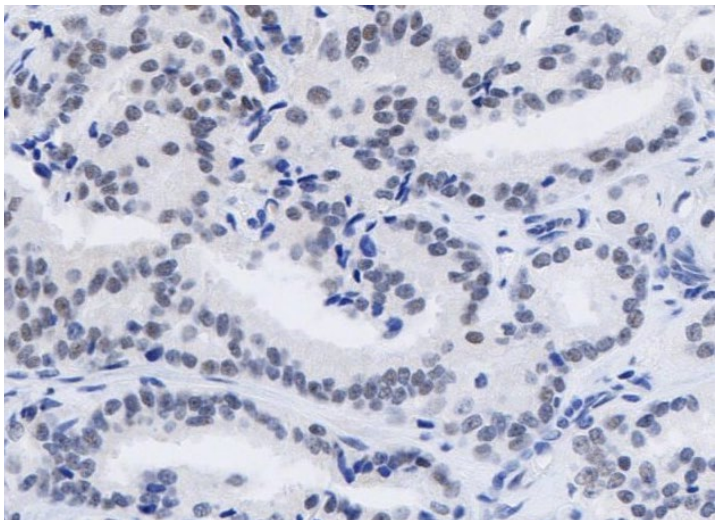
Bladder carcinoma



Renal cell carcinoma



Prostate adenocarcinoma



Prostate adenocarcinoma - Isotype control

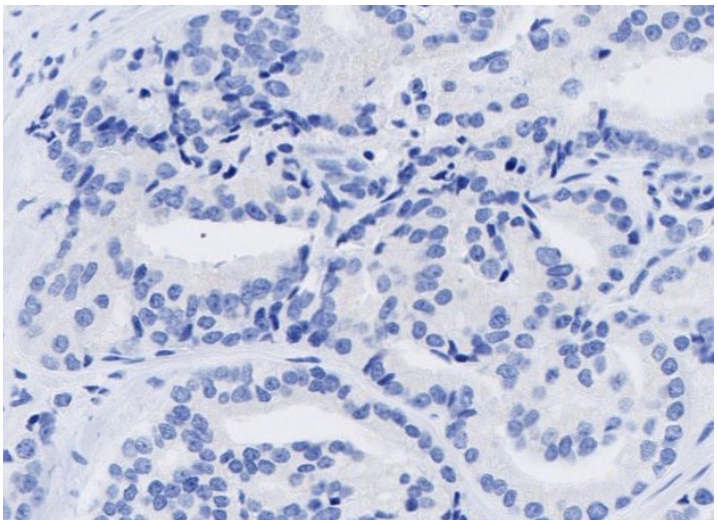


Figure 4. CDK12 expression in cancer. IHC staining of multi-cancer human tissues using anti-CDK12 (ab317746) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue.
Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

CDK12 expression in cancer (DISCOVERY ULTRA)

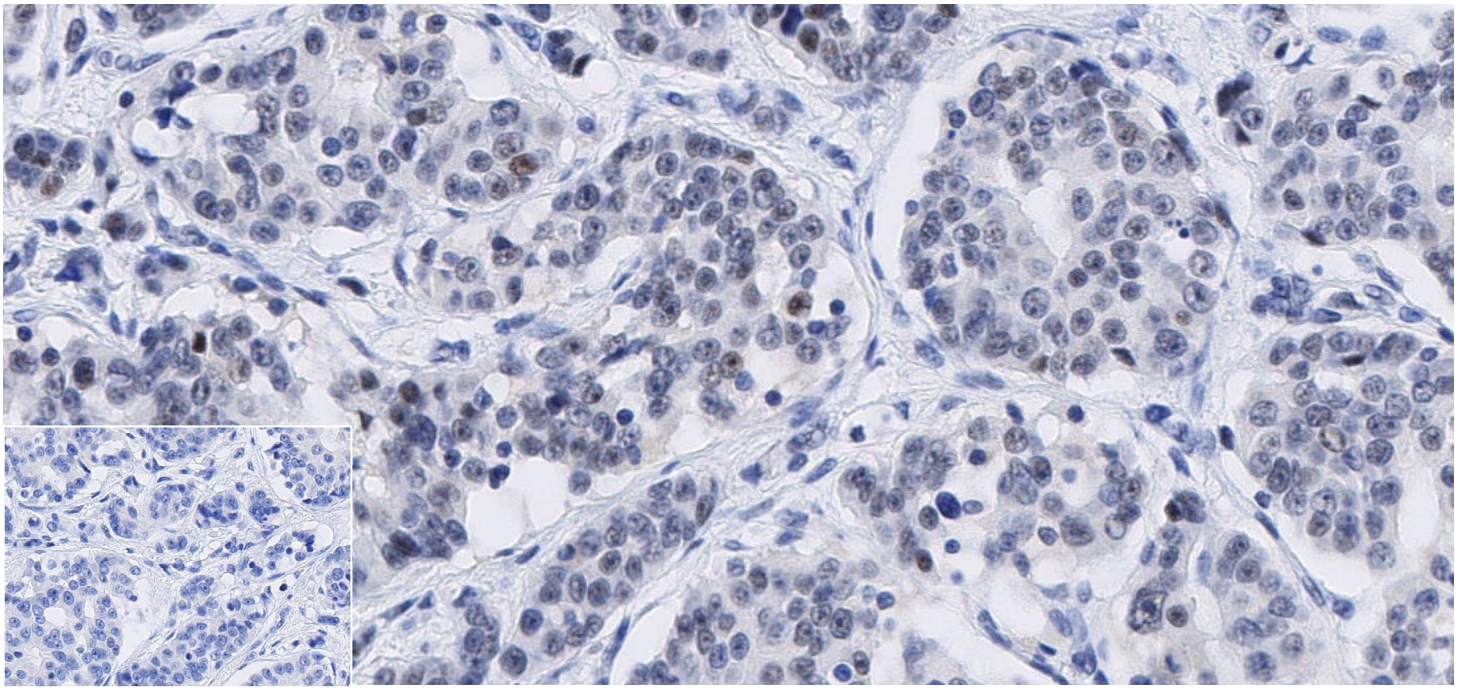
Donor	Diagnosis	CDK12 average expression intensity score
#1	Breast cancer	2+
#2	Breast cancer	3+
#3	Breast cancer	3+
#4	Breast cancer	3+
#5	Breast cancer	1+
#6	Breast cancer	3+
#7	Normal breast	3+
#8	Ovarian cancer	2+
#9	Ovarian cancer	2+
#10	Normal ovary	0
#11	Prostate cancer	0
#12	Prostate cancer	0
#13	Prostate cancer	1+
#14	Prostate cancer	0
#15	Prostate cancer	3+
#16	Prostate cancer	3+
#17	Normal prostate	0
#18	Normal prostate	1+

Table 4. Summary of CDK12 expression in the cancer and normal tissues. The staining intensity of tumor cells (disease samples) or normal cells (normal samples) was evaluated by eye and given a average score of 0-3+ (0 = negative cells; 1+ = weak stained cells; 2+ = moderate stained cells; 3+ = strong stained cells). The IHC images of donor #1-16 are shown in Figures 5-7 (DISCOVERY ULTRA).

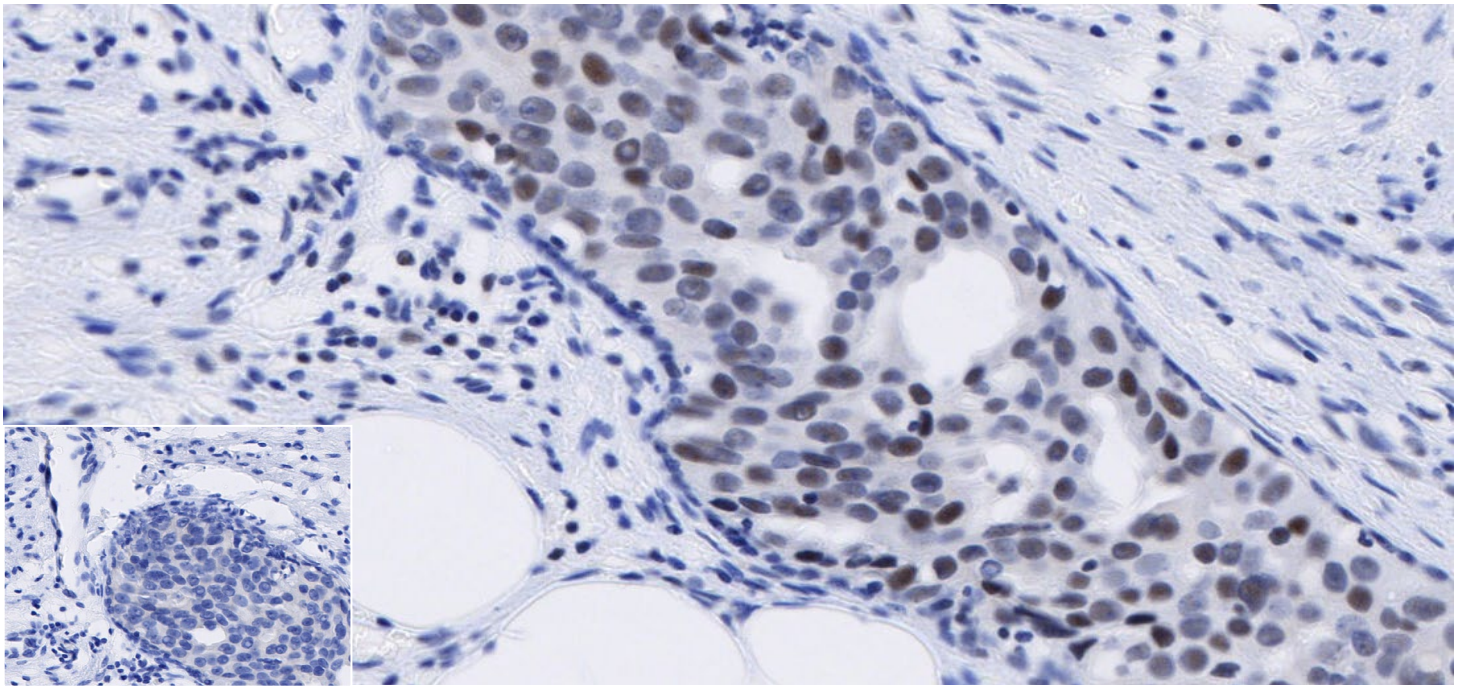
CDK12 expression in breast cancer (DISCOVERY ULTRA)

Below are representative images from individual cases of breast cancer, showing CDK12 expression.

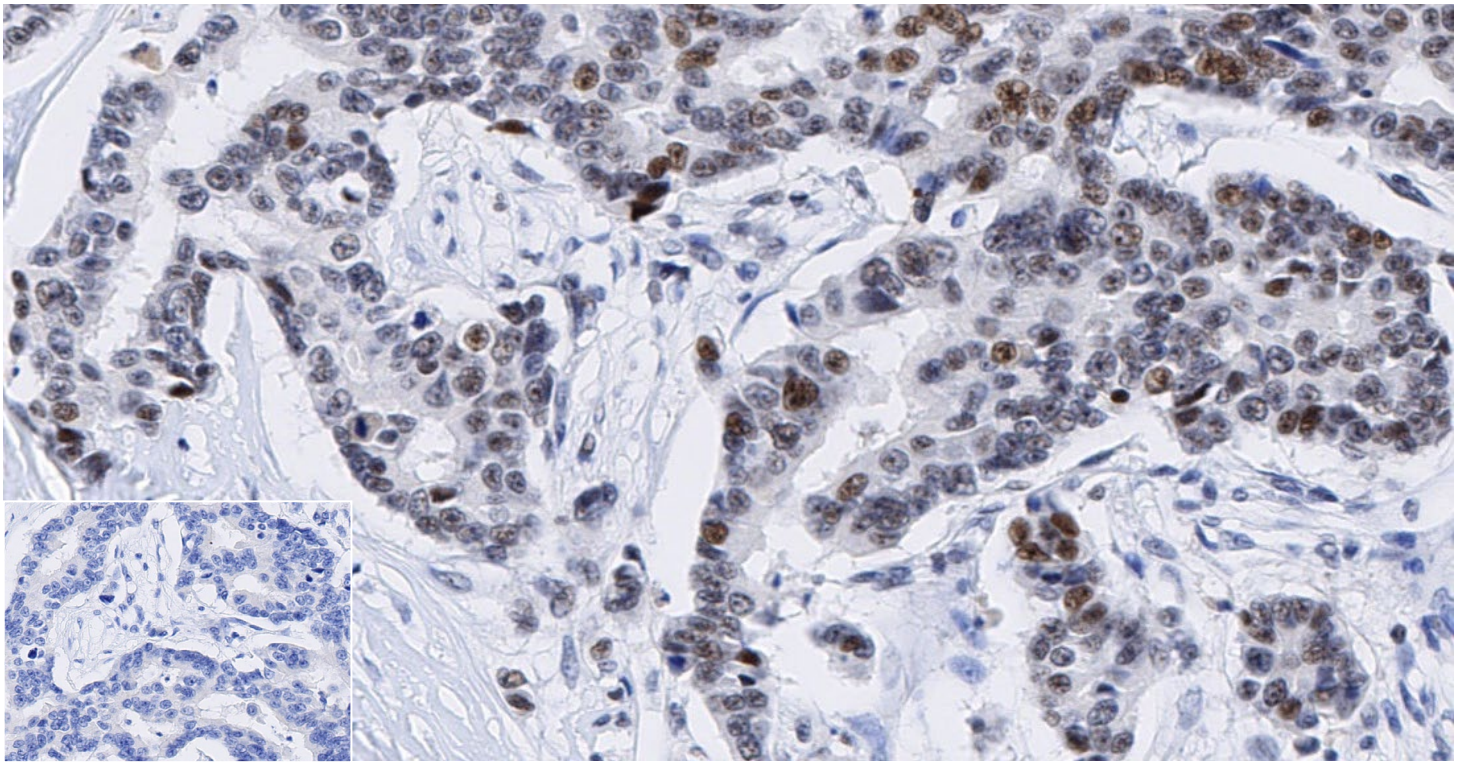
CDK12 (2+)



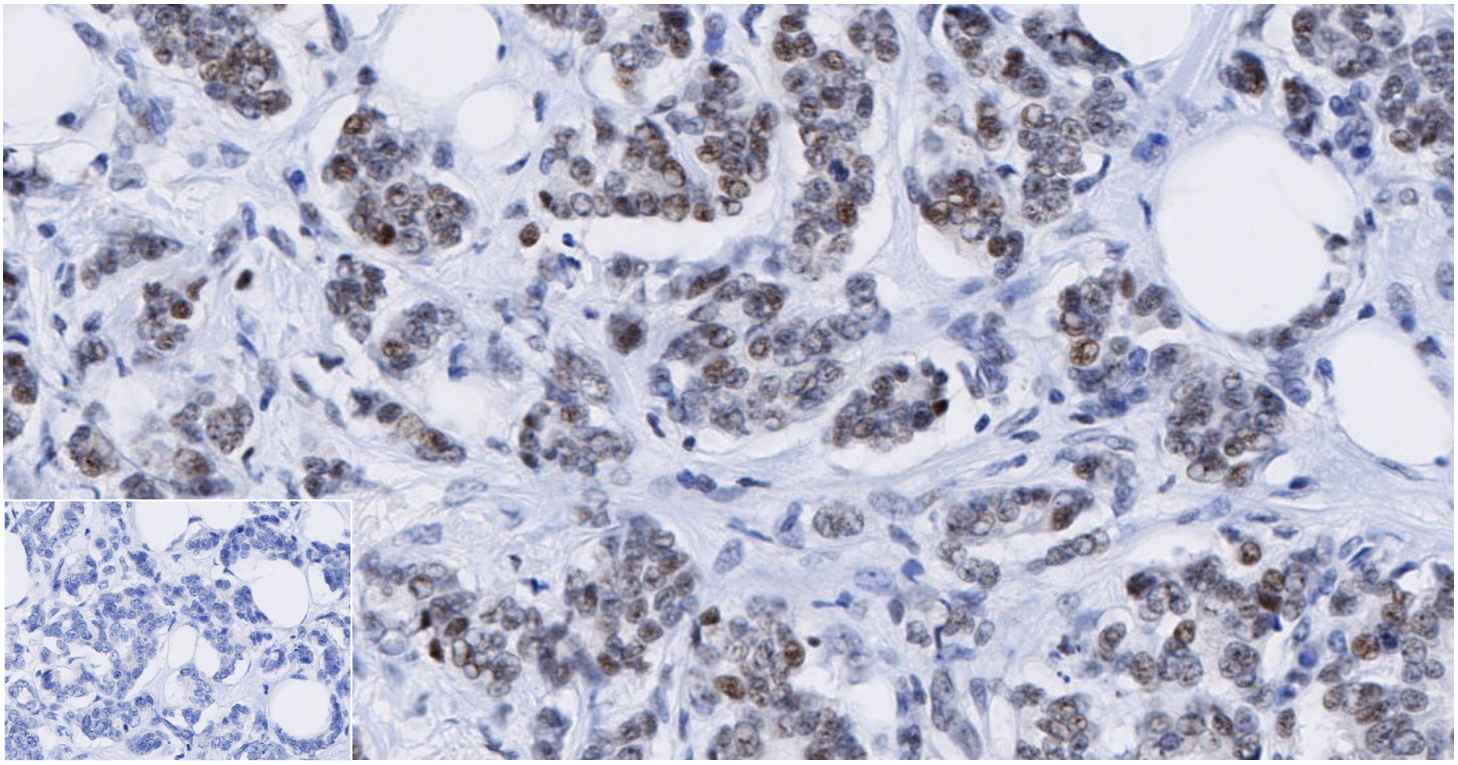
CDK12 (3+)



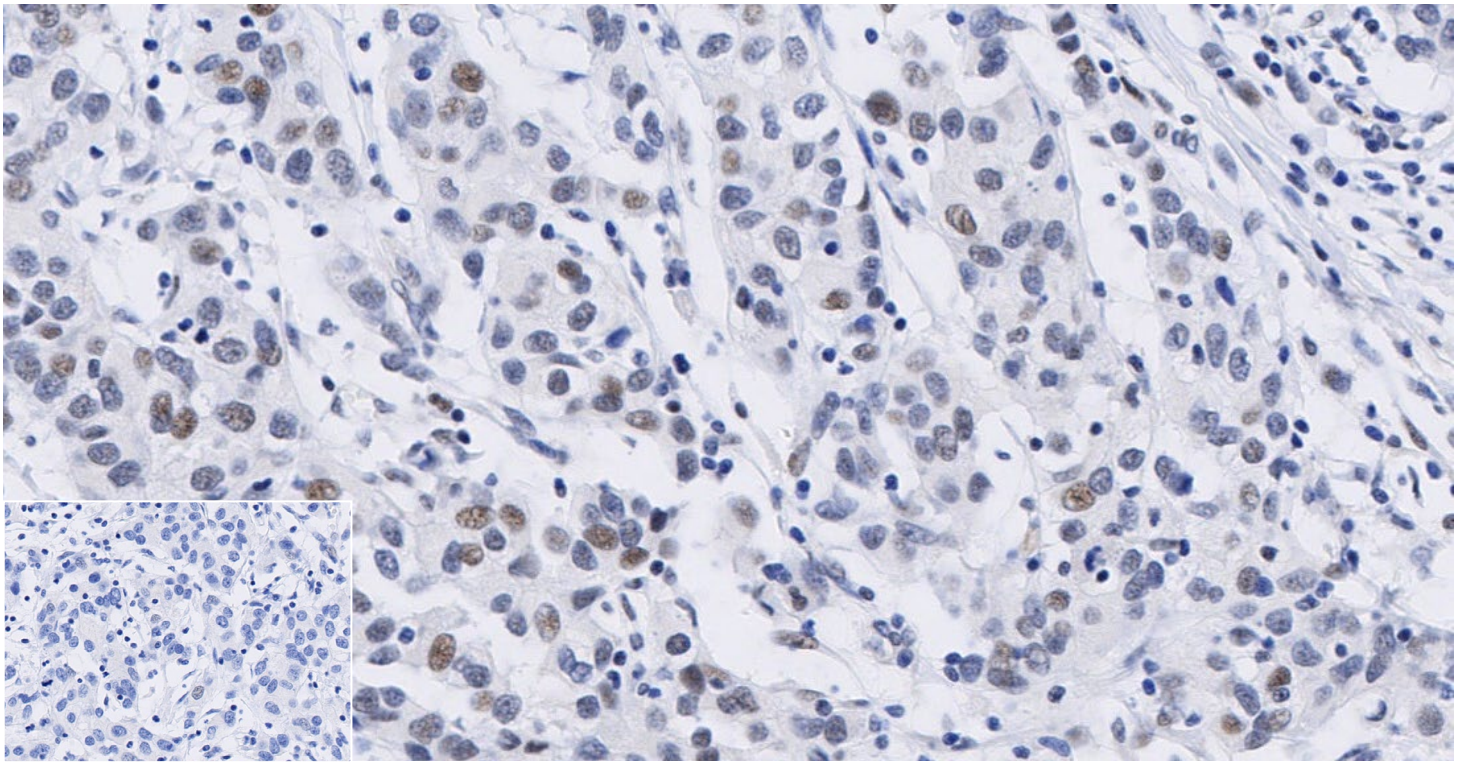
CDK12 (3+)



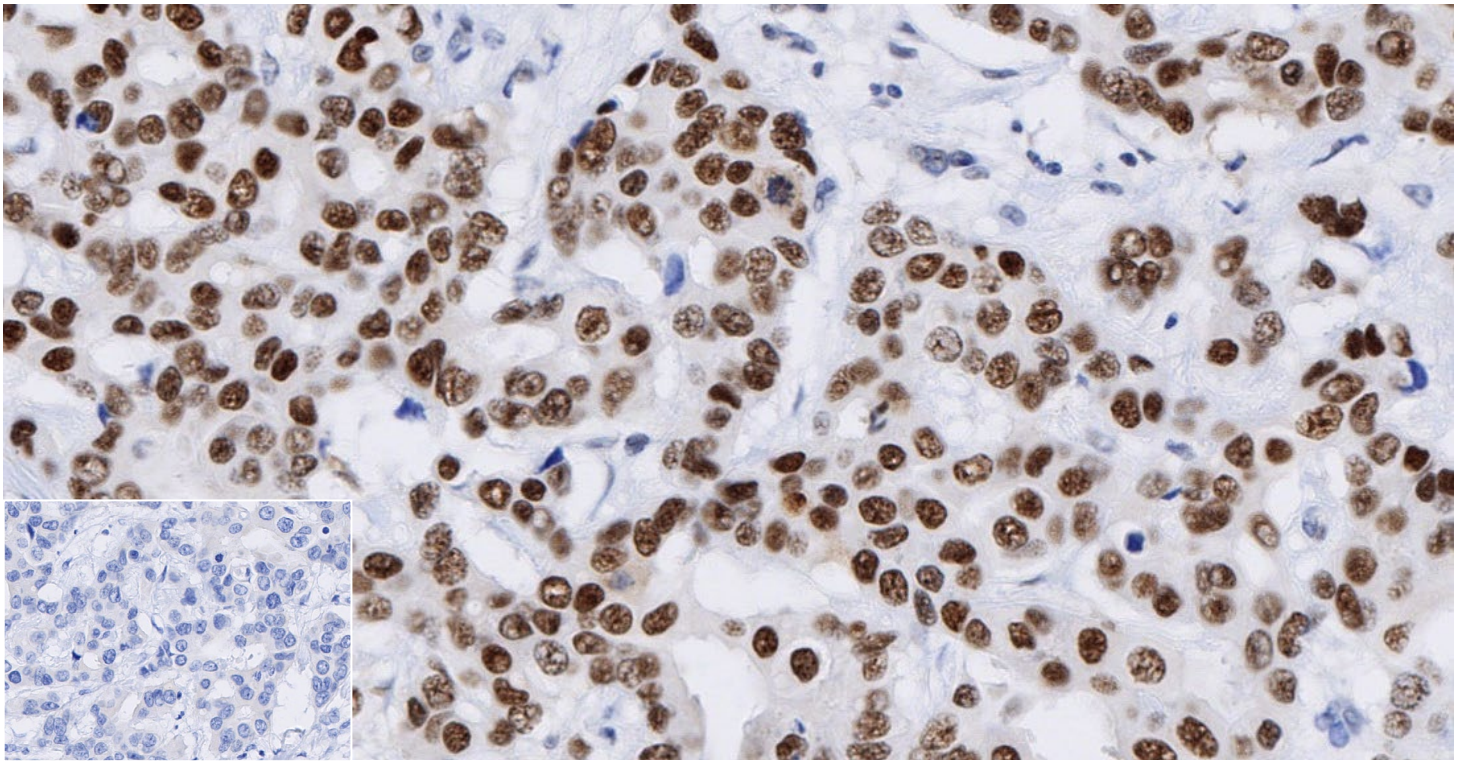
CDK12 (3+)



CDK12 (1+)



CDK12 (3+)



CDK12 (3+)

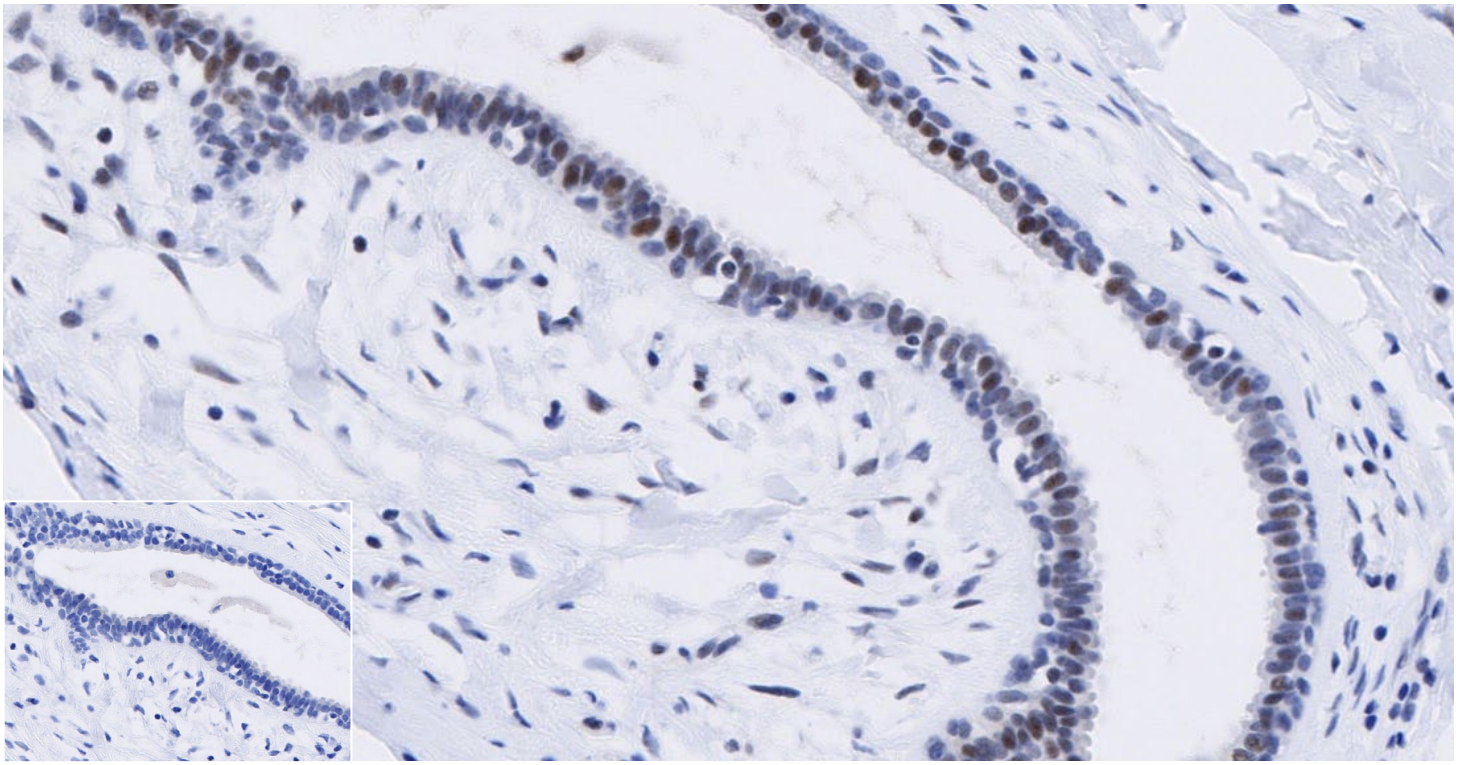
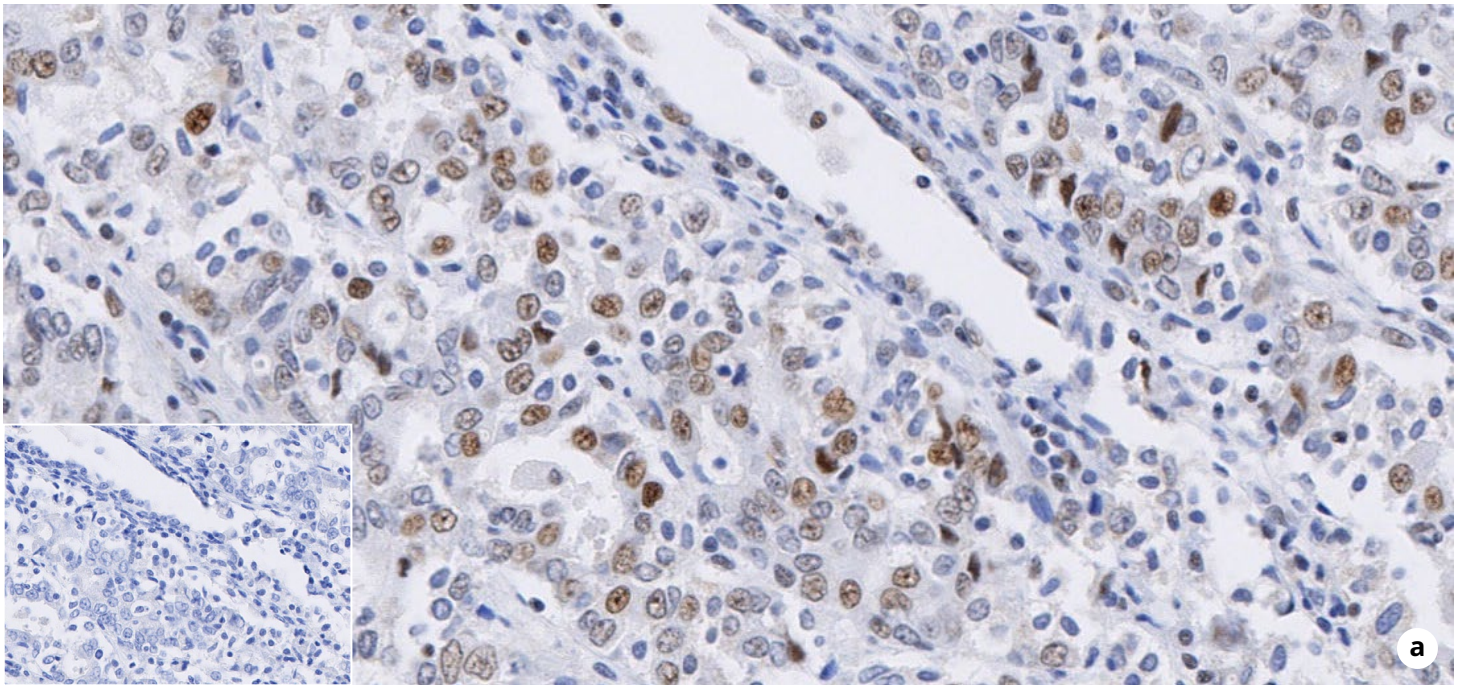


Figure 5. CDK12 expression in breast cancer. IHC staining of breast cancer tissues using anti-CDK12 (ab317746) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730) as insets. Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

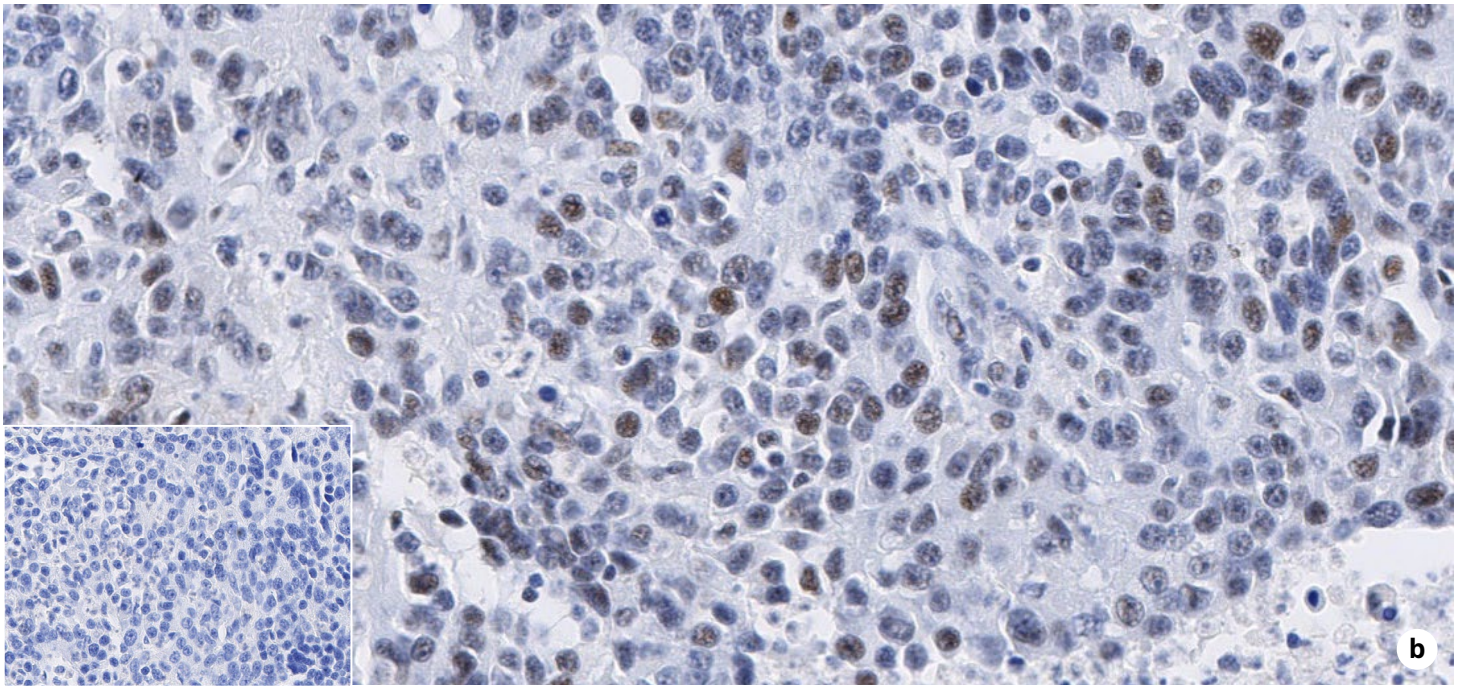
CDK12 expression in ovarian cancer (DISCOVERY ULTRA)

Below are representative images from individual cases of ovarian cancer (a-b) and normal ovarian (c), showing CDK12 expression.

CDK12 (2+)



CDK12 (2+)



CDK12 (0)

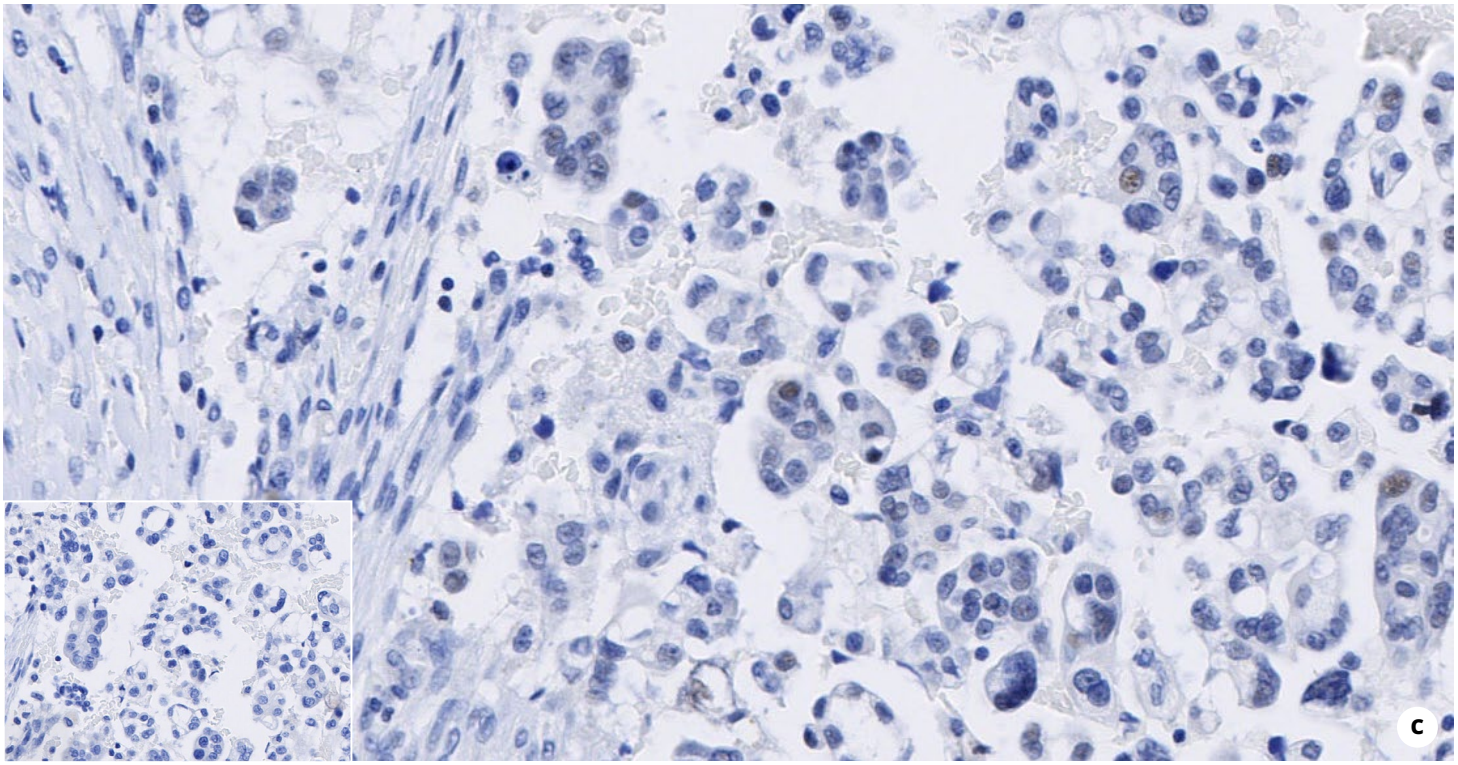
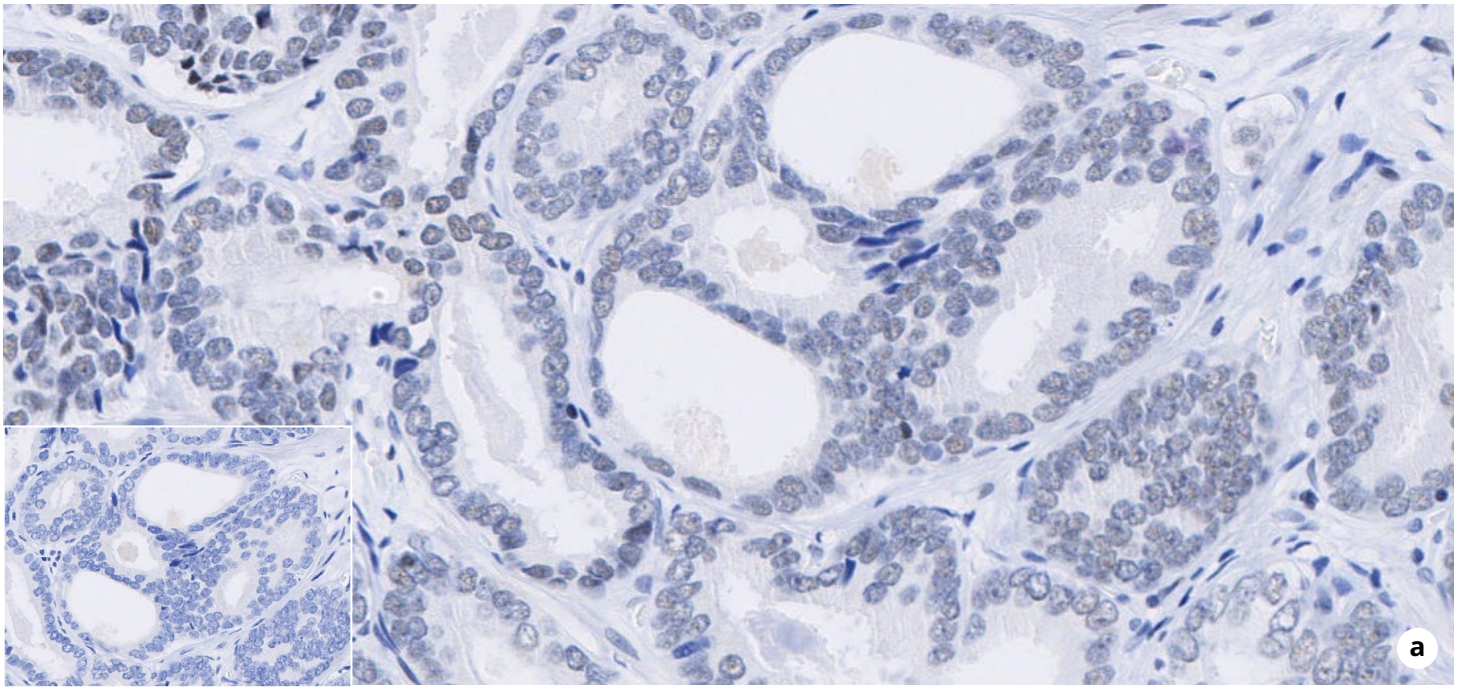


Figure 6. CDK12 expression in ovarian cancer. IHC staining of ovarian cancer tissues using anti-CDK12 (ab317746) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730) as insets. Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

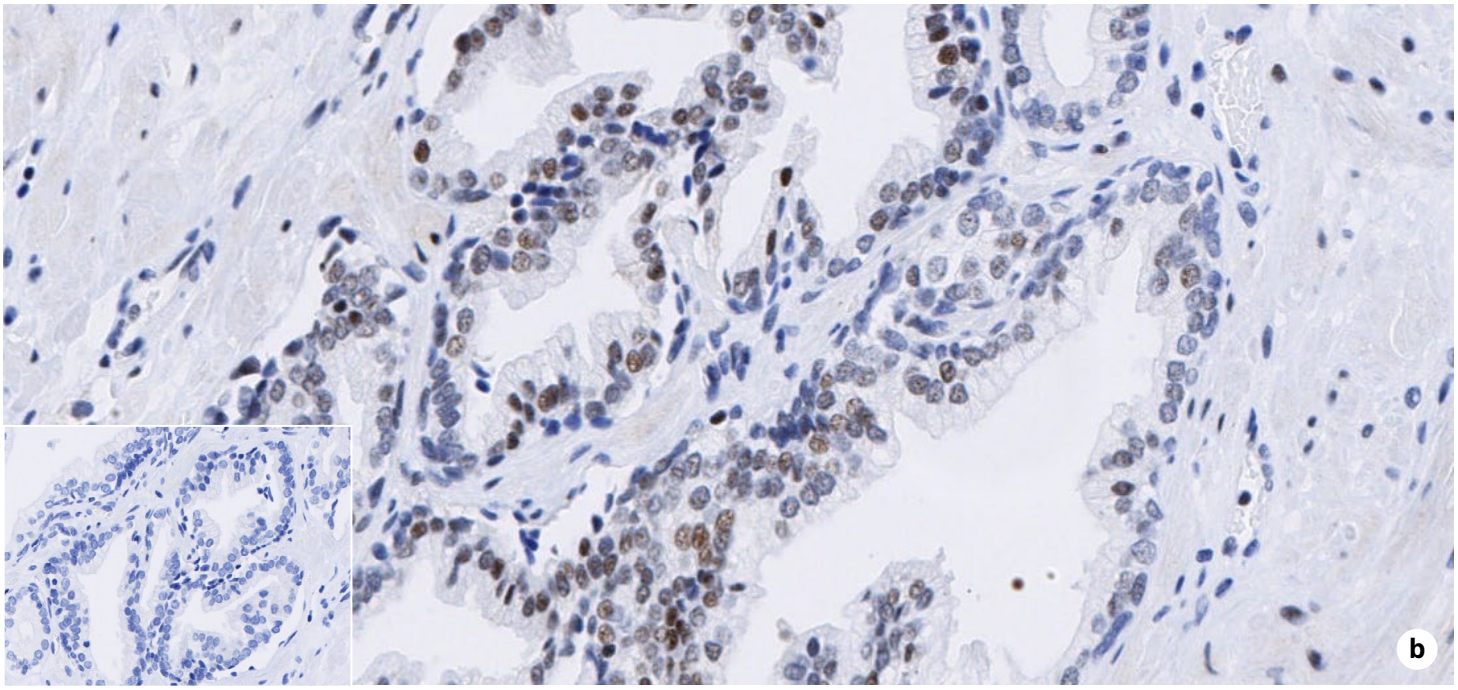
CDK12 expression in prostate cancer (DISCOVERY ULTRA)

Below are representative images from individual cases of prostate cancer (a-d) and normal prostate (e-f), showing CDK12 expression.

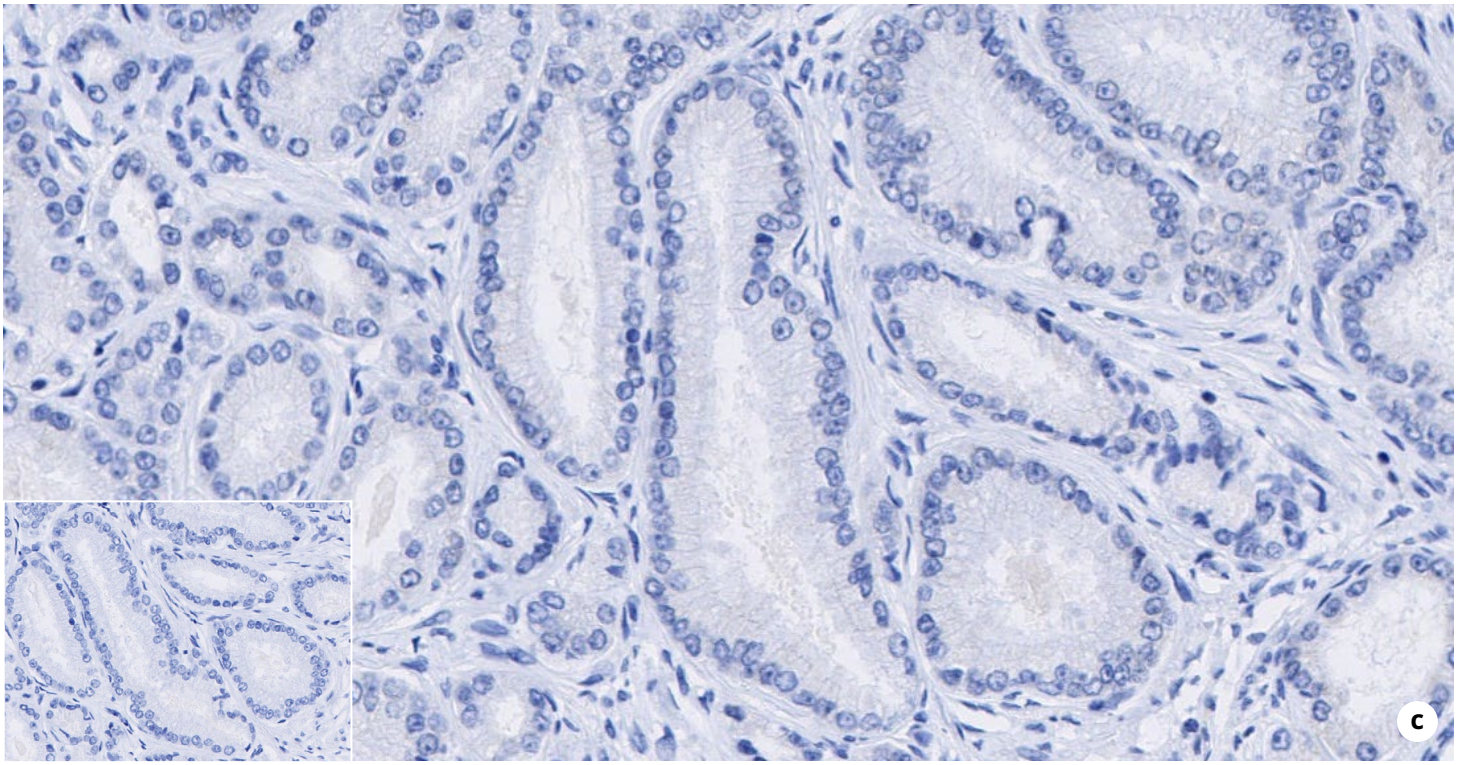
CDK12 (1+)



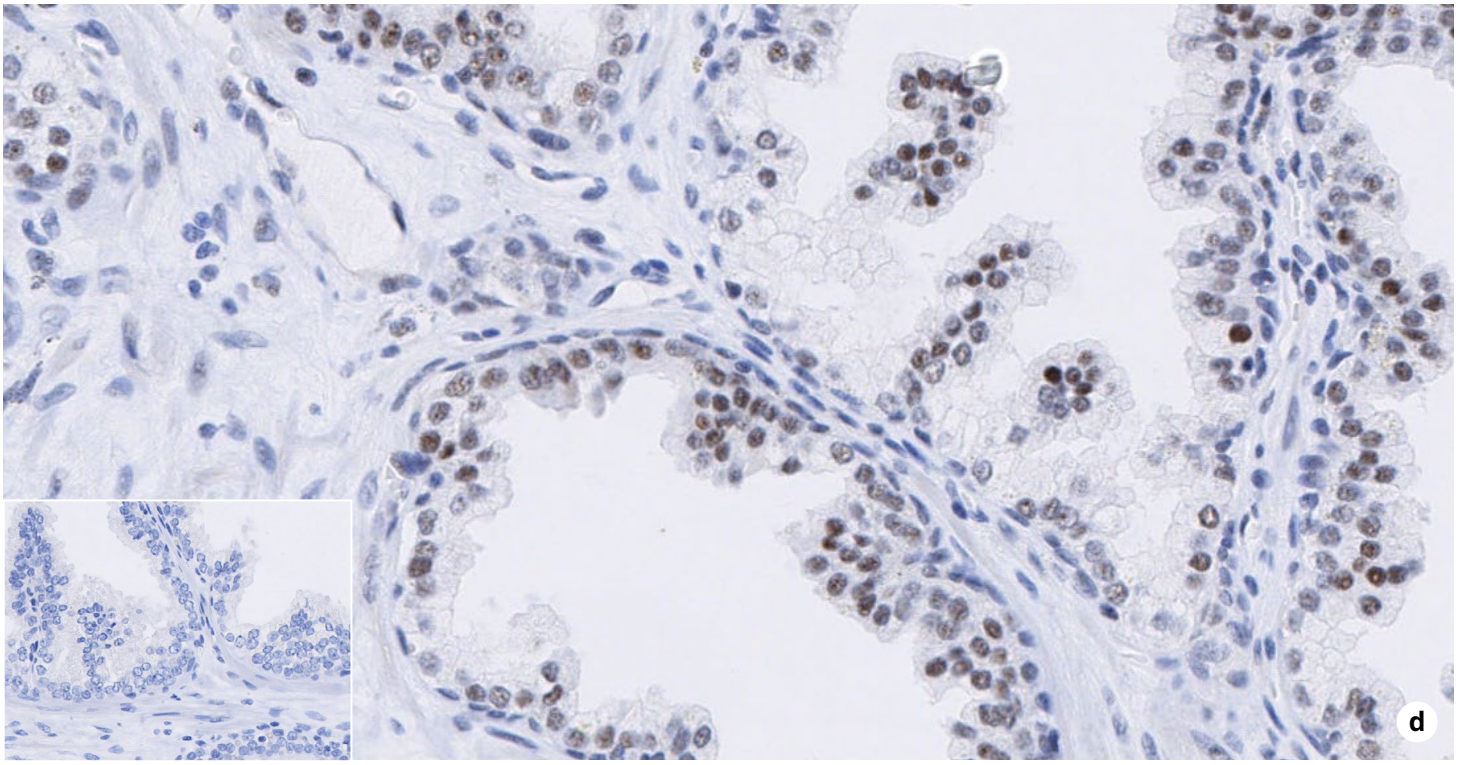
CDK12 (3+)



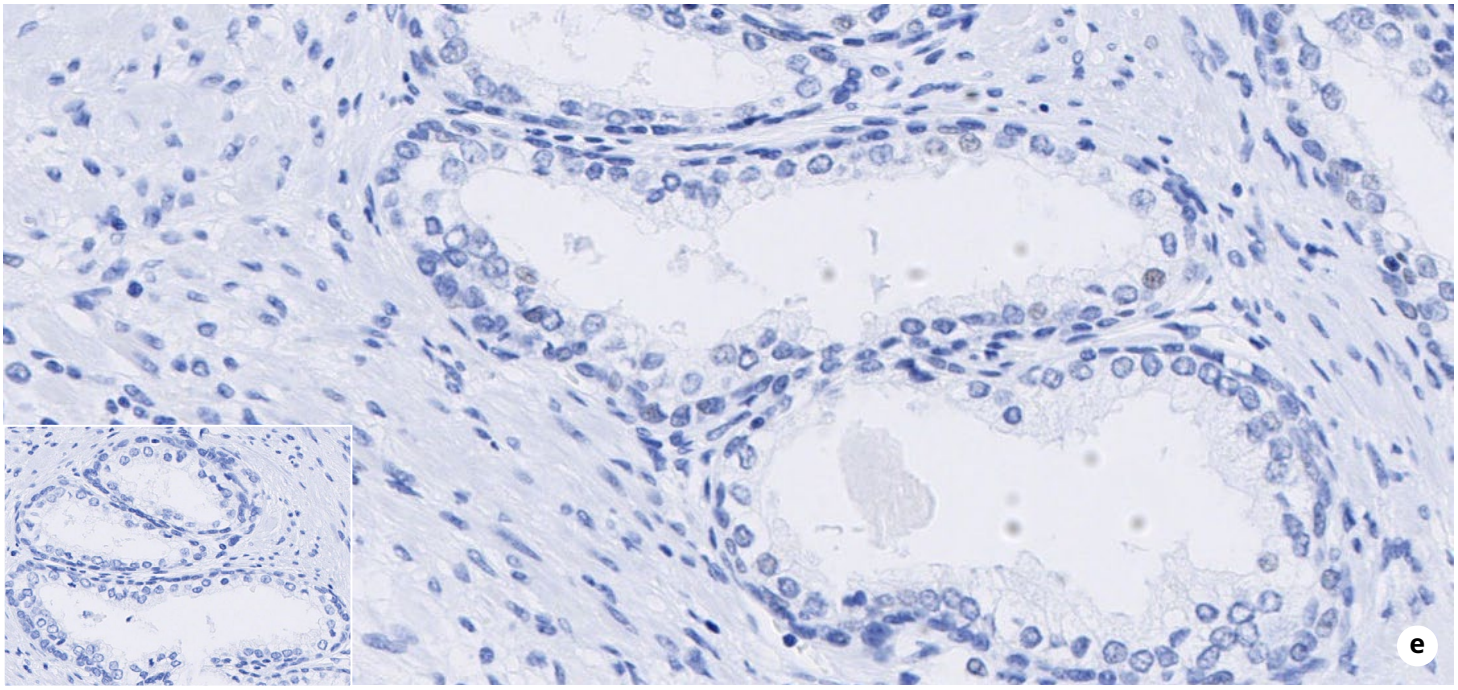
CDK12 (0)



CDK12 (3+)



CDK12 (0)



CDK12 (1+)

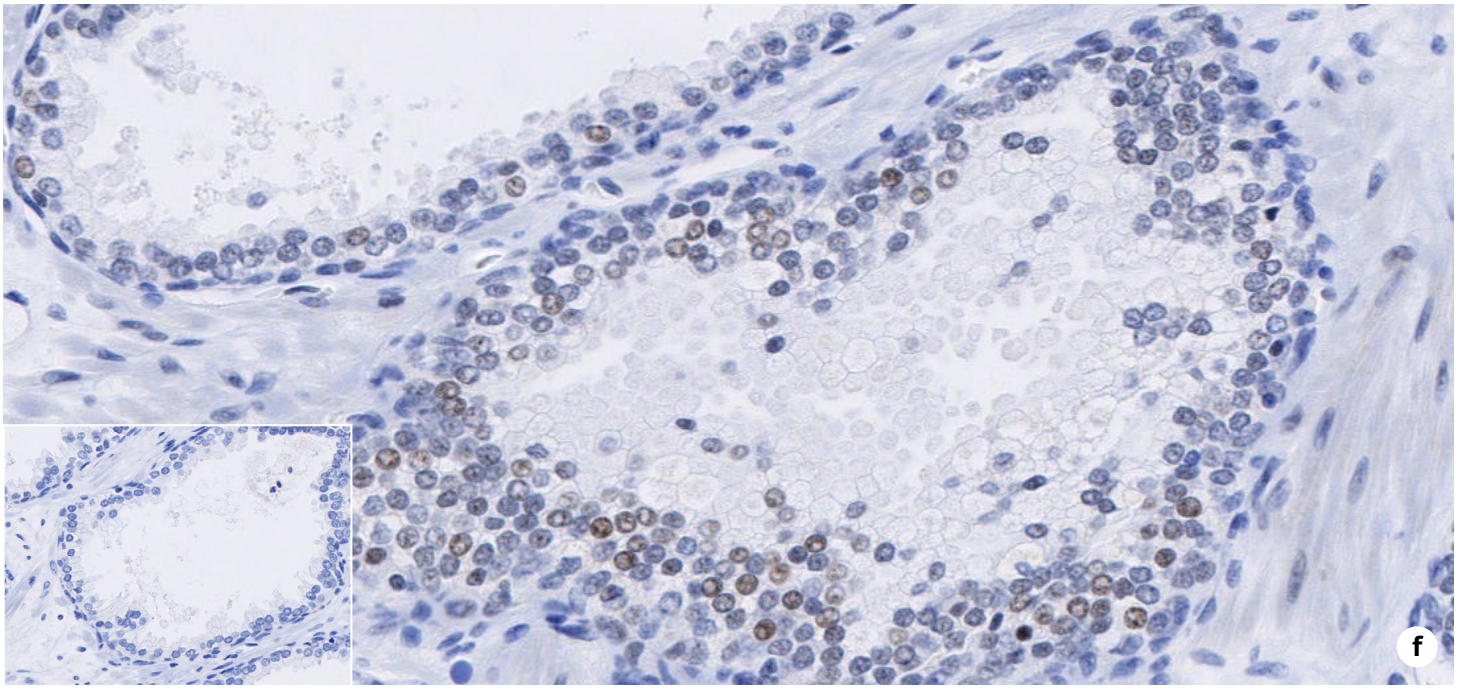


Figure 7. CDK12 expression in prostate cancer. IHC staining of prostate cancer tissues using anti-CDK12 (ab317746) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730) as insets. Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on NanoZoomer S360 (Hamamatsu Photonics K.K.) and imaged at 20X on Aperio® ImageScope.

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