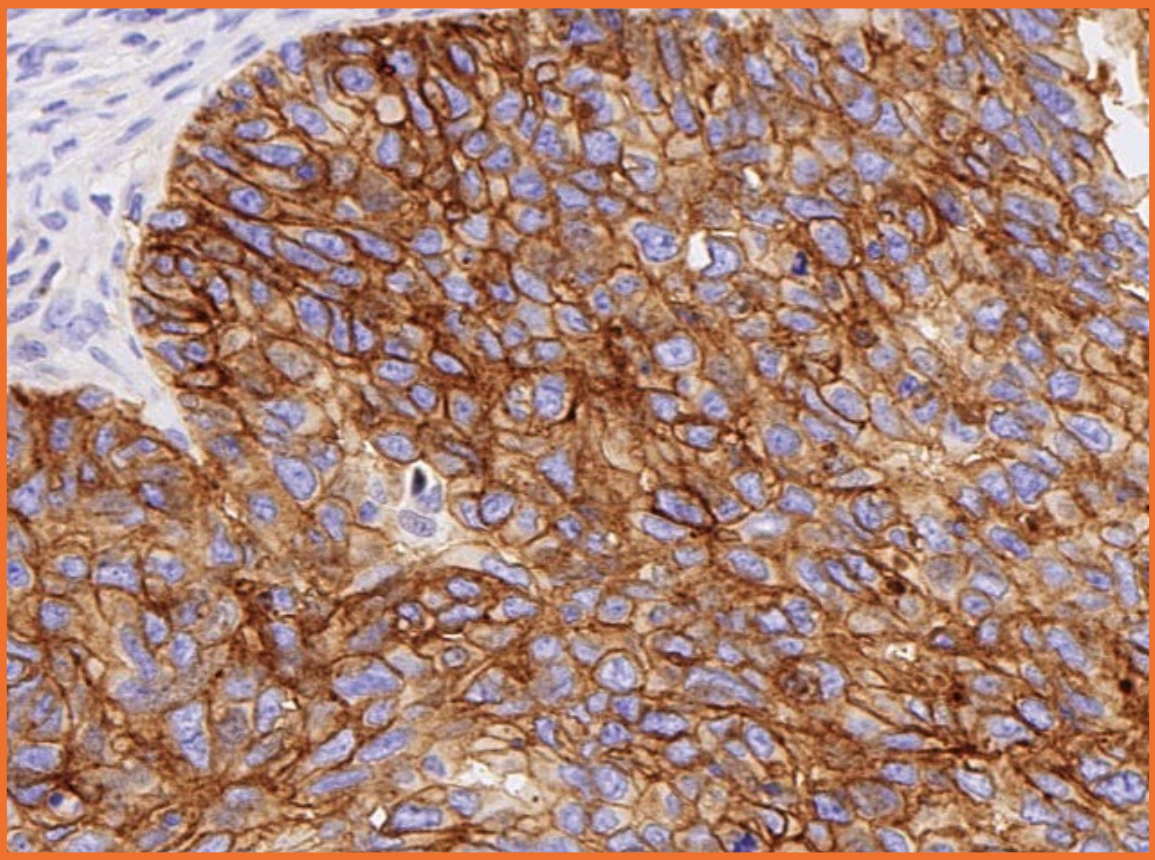
Enhanced validation data

Anti-CD47 recombinant antibody – ab218810



CD47 expression in ovarian serous cystadenocarcinoma

progress happens together

abcam

AB0587_v1.0_120425

Enhanced validation of Anti-CD47 recombinant antibody [EPR21794] – ab218810

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 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-CD47 antibody (ab218810) 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 CD47 expression was performed using the artificial intelligence (AI)-driven digital image analysis software Visiopharm® (Visiopharm A/S).

AB0587_v1.0_120425

Target overview

HGNC symbol
CD47

Approved name
CD47 molecule

Chromosomal location
3q13.12

Function

CD47 is a transmembrane protein receptor that interacts with multiple ligands to regulate a range of cellular and metabolic processes, including immune checkpoint signaling, apoptosis, cellular stress responses, proliferation, and metabolic activity[1].

CD47 functions as an immune checkpoint by interacting with its ligand SIRPα to transmit an inhibitory "don't eat me" signal to macrophages, preventing the phagocytosis of healthy cells[2].

Tissue specificity

CD47 is widely expressed in most normal tissues, including hematopietic cells, brain, lung and kidney[3]. This broad expression pattern is consistent with its role in multiple physiological processes.

Overexpression of CD47 is in several cancers including ovarian[4], hematological and lymphoma[5] and NSCL cancer[6] permitting cancer cell immune evasion. CD47 has become a promising therapeutic target[2,5,7,8, 9, 10] and can serve as prognostic marker[8].

Cellular localization

Cell membrane

Database links

Entrez Gene: 4609
OMIM®: 601028
Uniprot: Q08722

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[11-14].

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	5	97	Quickarrays (#OVC1021)
Lung cancer	102	102	5	97	Quickarrays (#LUC1021)
Lymphoma	102	102	5	97	Quickarrays (#LYM1021)

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 one donors: 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, ovaria carcinoma, cervical cancer, head and neck carcinoma and endometrial cancer. The following tissues were from single donors: lung (squamous cell carcinoma (SCLC) and non-squamous cell carcinoma (NSCLC)), colon (adenocarcinoma and invasive adenocarcinoma), breast (ductal carcinoma and invasive lobular carcinoma), B cell lymphoma, T cell lymphoma, gliomas (grade II and IV) and placenta.

Step	Reagents	Method
Deparaffinization	DISCOVERY Wash (RUO)	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	32 min, 100 °C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Anti-CD47 recombinant antibody [EPR21794] – ab218810 diluted in Discovery antibody diluent (#760-108) to a final concentration of 1 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-CD47 recombinant antibody [EPR21794] – ab218810 diluted in Bond™ primary antibody diluent (#AR9352) to a 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
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.

H-score analysis

A quantitative H-score analysis of CD47 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, artefact exclusion and segmentation (where relevant) 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, cell membrane H-scores of either tumor cells in the ovarian and lung TMAs or whole core in the lymphoma TMA were calculated in Visiopharm® and the graphical representation was generated using GraphPad Prism 10.

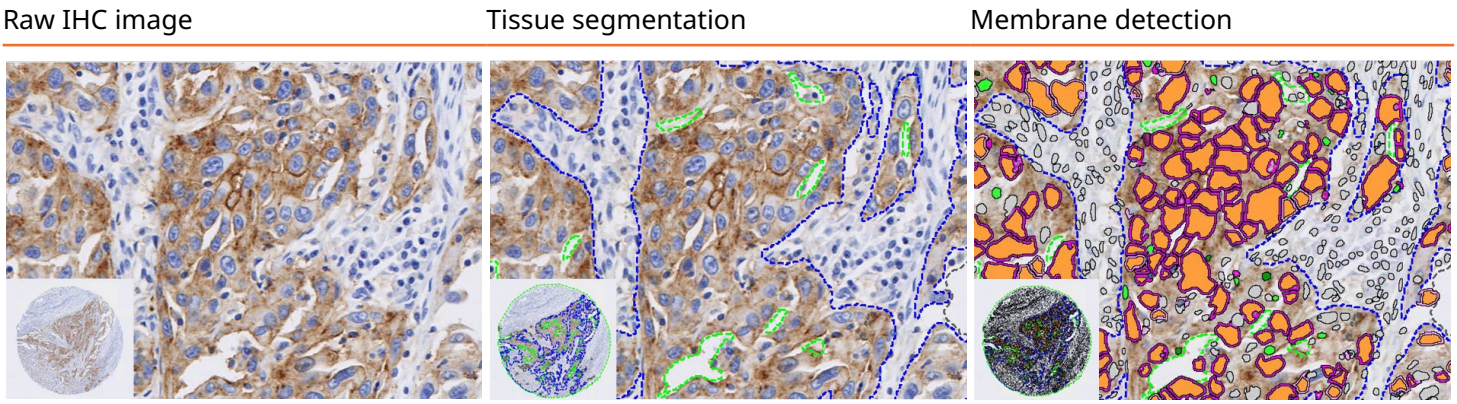


Figure 1. Visiopharm® tissue segmentation and membrane detection. Raw IHC images were subjected to total cell detection to determine staining intensity. There are four intensity scores shown in the example image: gray (0), pink (1+), magenta (2+) and purple (3+). Areas in orange are irrelevant to these analyses.

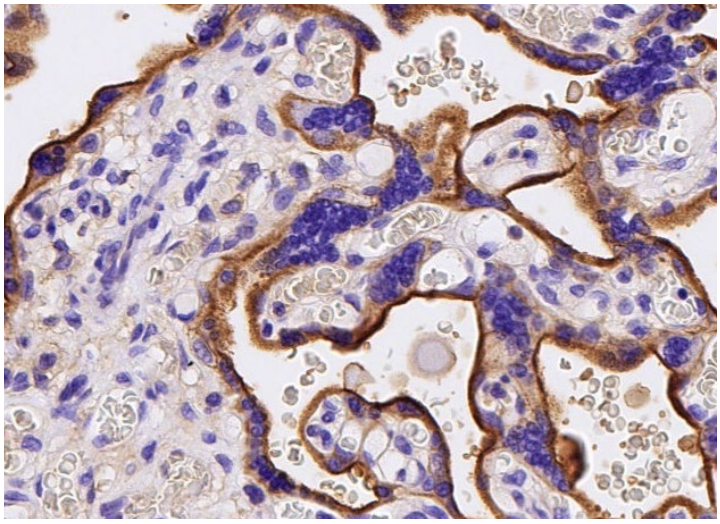
IHC staining	Corresponding intensity score	Visiopharm® intensity threshold
Negative	0	> 255
Weak	1+	< 255
Moderate	2+	< 190
Strong	3+	< 160

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.

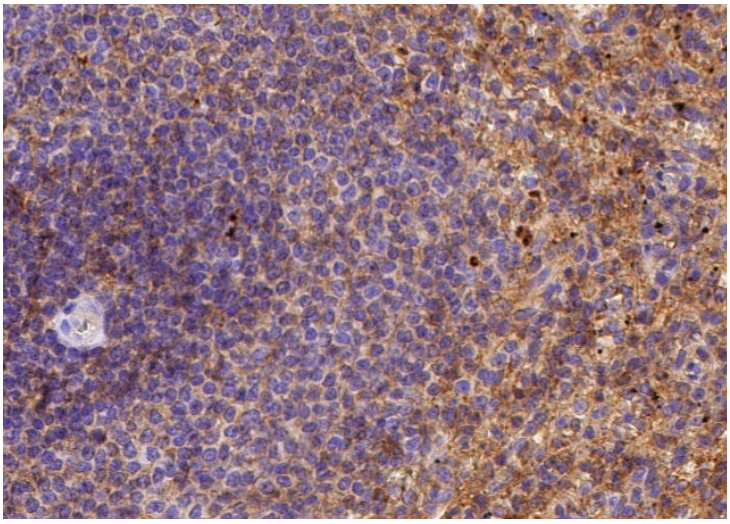
CD47 expression in multi-normal TMA (BOND™ RX)

Below are the representative images of selected tissues from the multi-normal TMA. CD47 expression was detected in the placenta, spleen, cerebrum, prostate, lung stomach, and tonsil, whereas low to no expression was observed in the kidney, heart, pancreas and skeletal muscle tissues.

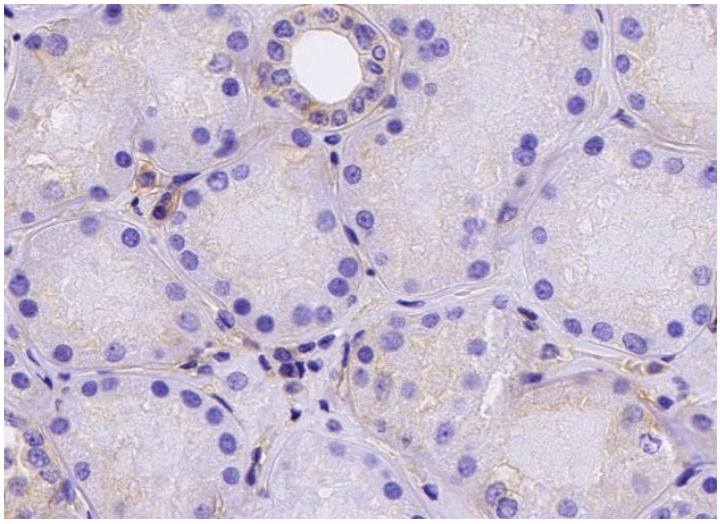
Placenta



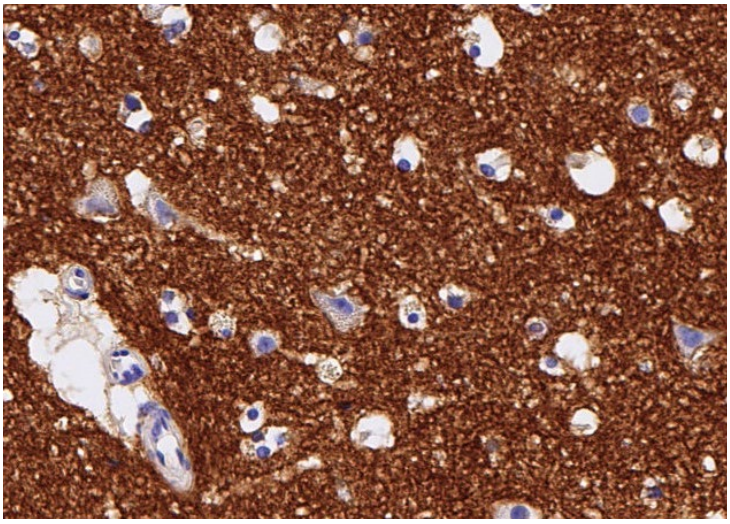
Spleen



Kidney

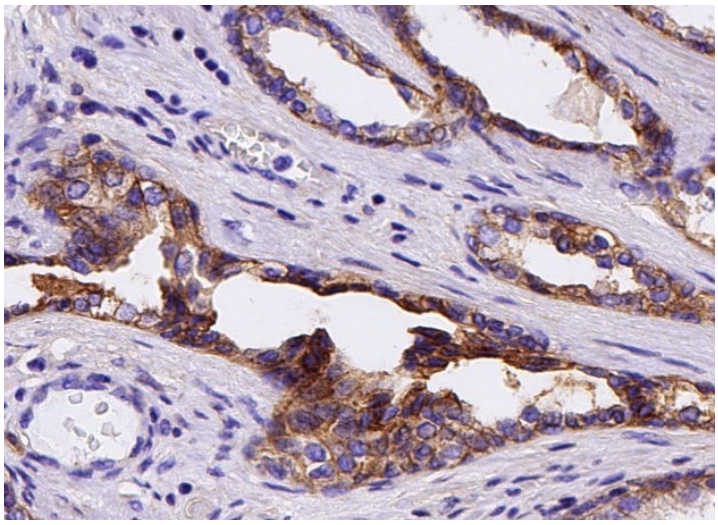


Cerebrum

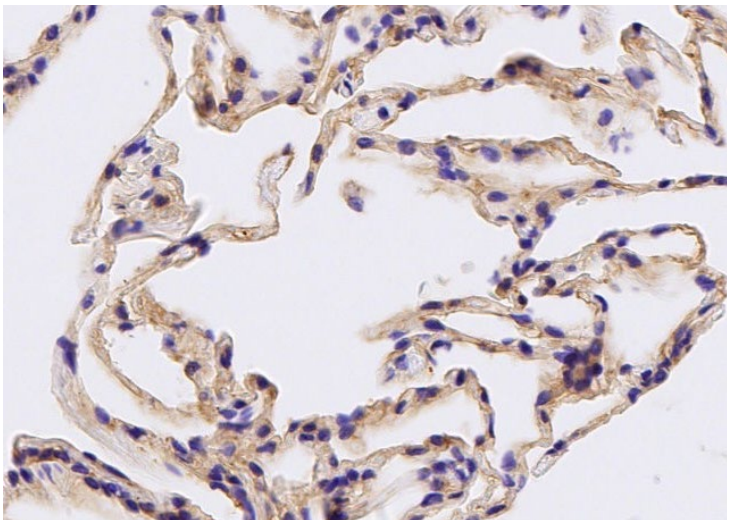


Enhanced validation data

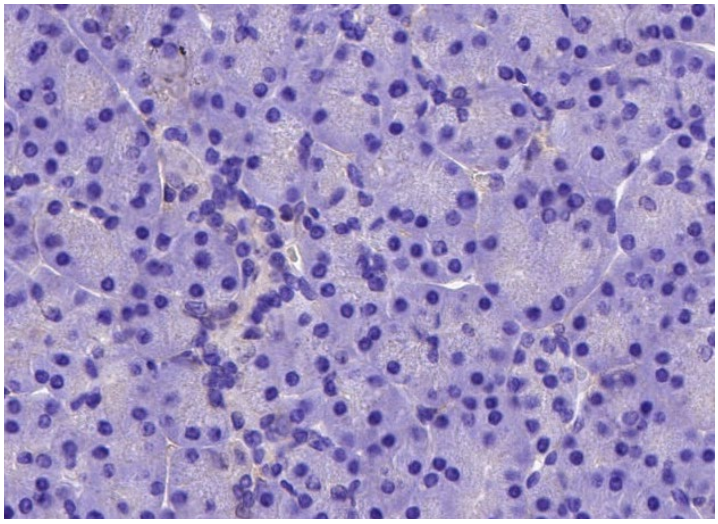
Prostate



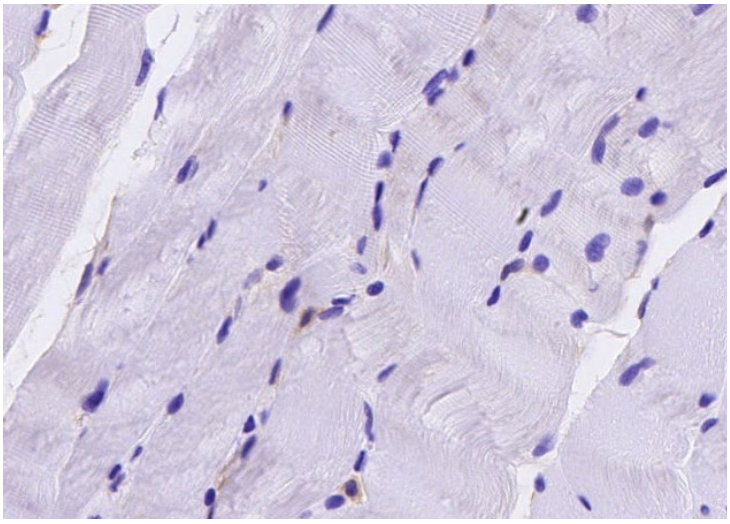
Lung



Pancreas

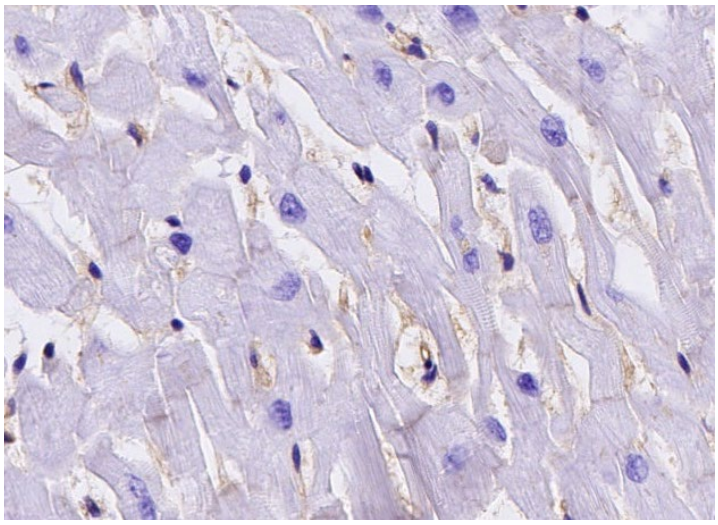


Skeletal muscle

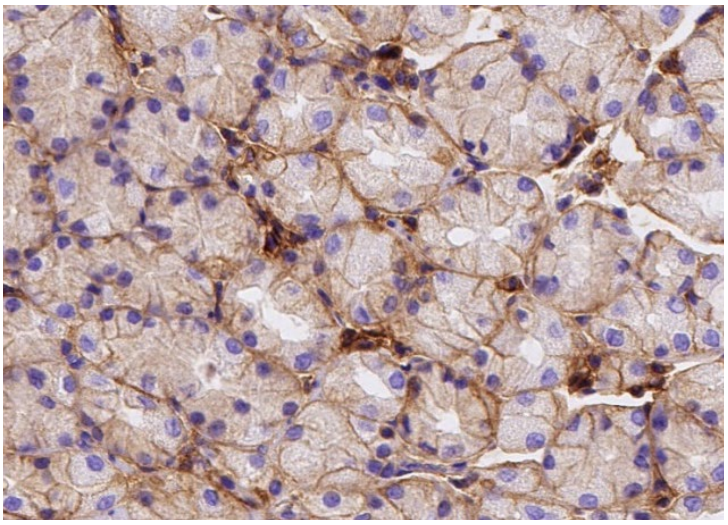


Enhanced validation data

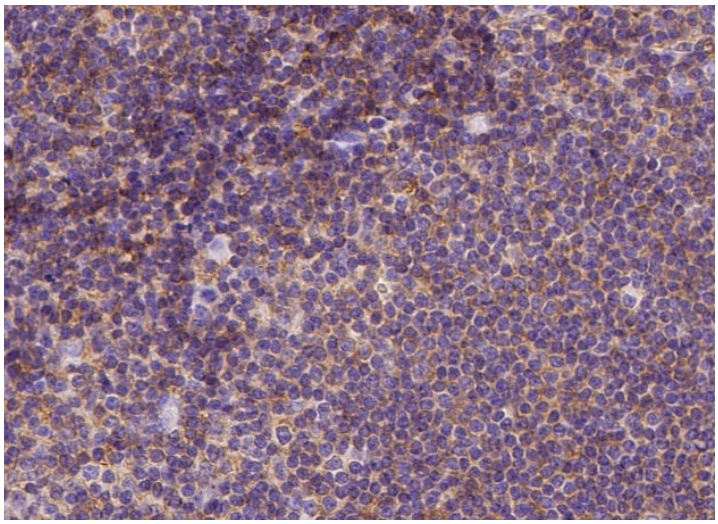
Heart



Stomach



Tonsil



Tonsil (isotype control)

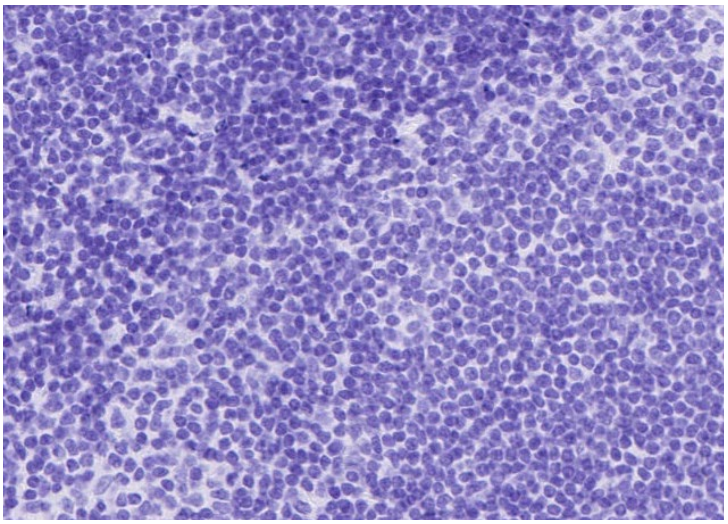


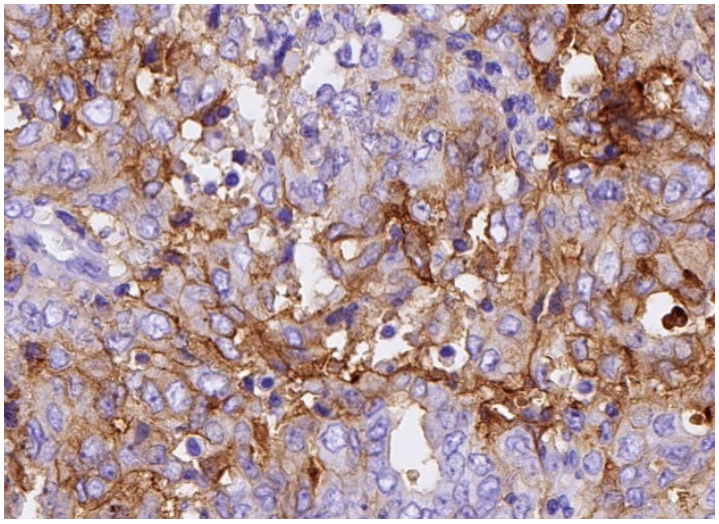
Figure 2. CD47 expression in human normal tissue. IHC staining of multi-normal human tissues using anti-CD47 (ab218810) (0.25ug/mL) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a BOND™ RX Research Stainer (Leica®), 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.

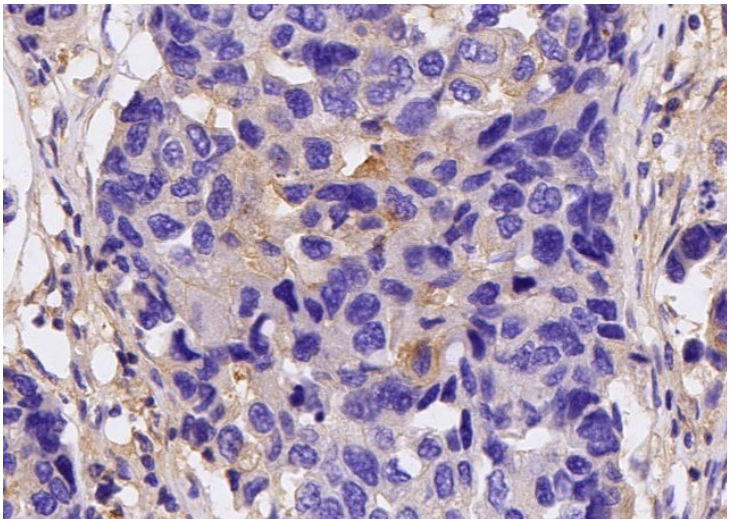
CD47 expression in multi-cancer TMA (BOND™ RX)

Below are the representative images of selected tissues from the multi-cancer TMA. CD47 expression was detected in ovarian carcinoma, breast ductal carcinoma, B cell lymphoma, T cell lymphoma, seminoma, glioblastoma, adenocarcinoma of the pancreas, lung, stomach and colon, whereas no expression was detected in hepatocellular carcinoma or head and neck carcinoma.

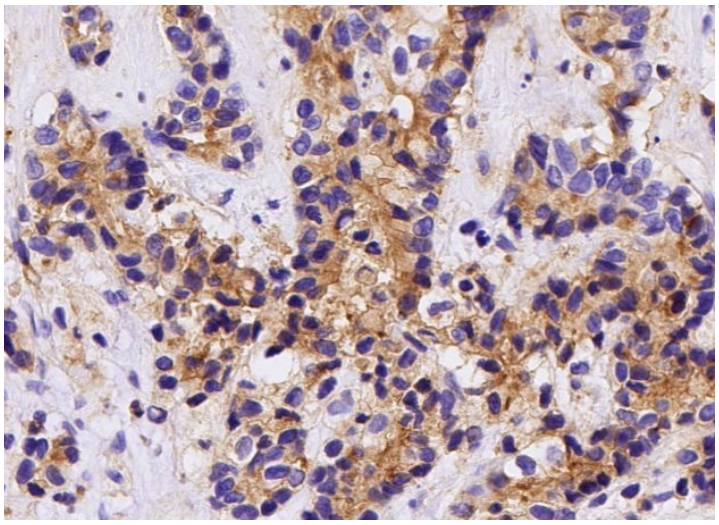
Ovarian carcinoma



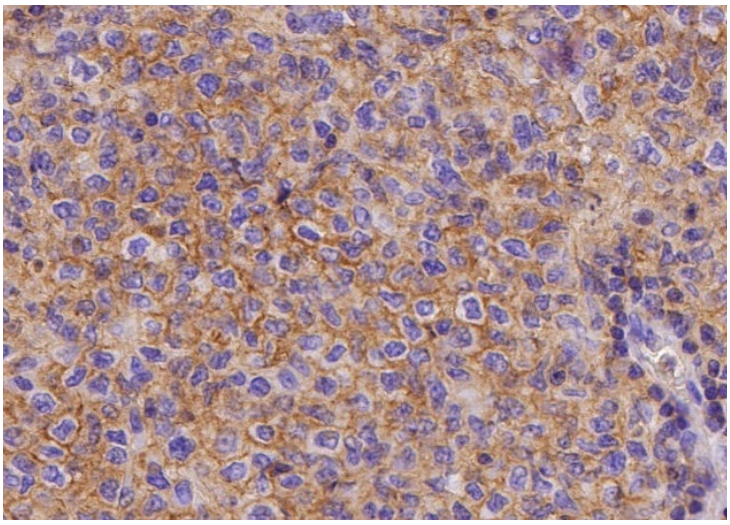
Breast ductal carcinoma



Pancreatic adenocarcinoma

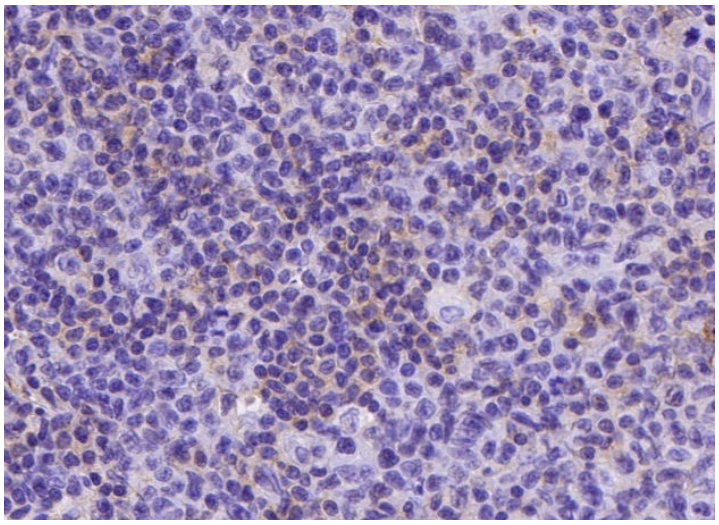


B cell lymphoma

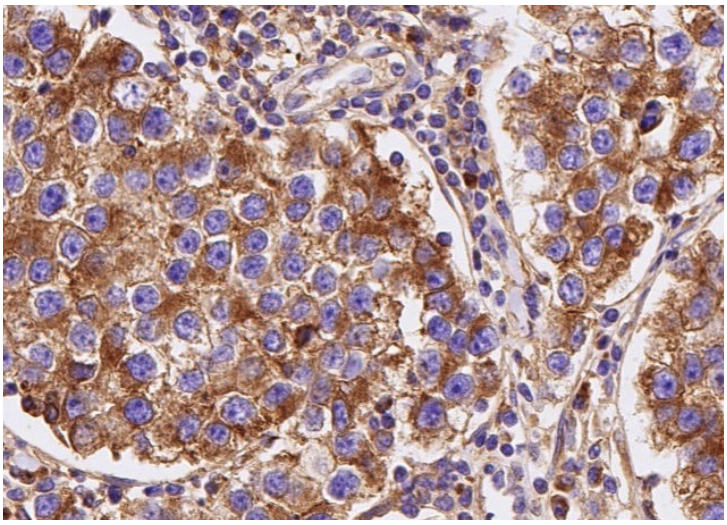


Enhanced validation data

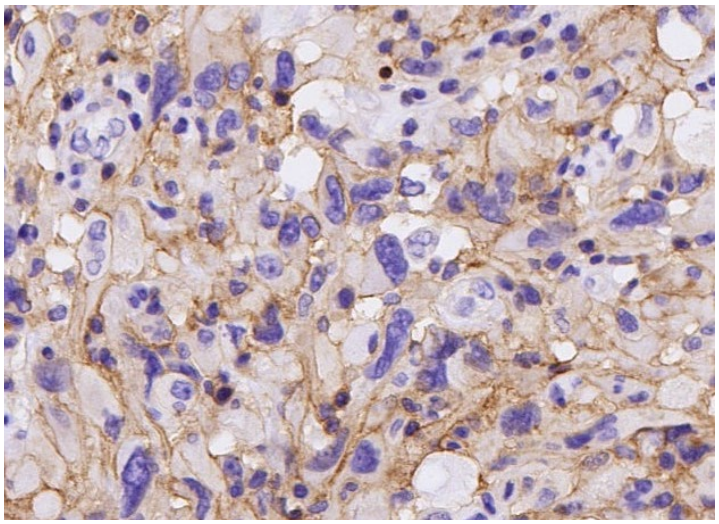
T cell lymphoma



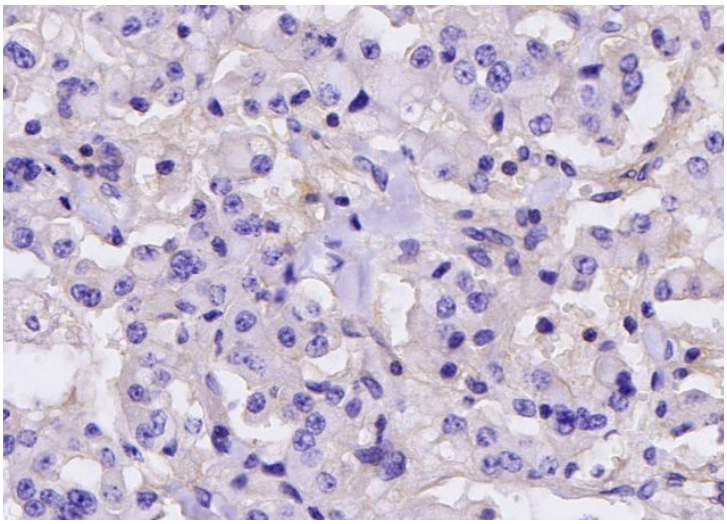
Testis seminoma



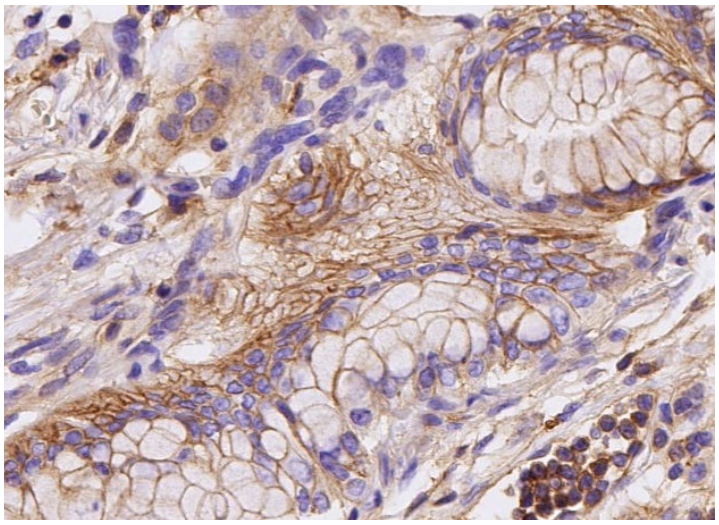
Glioblastoma



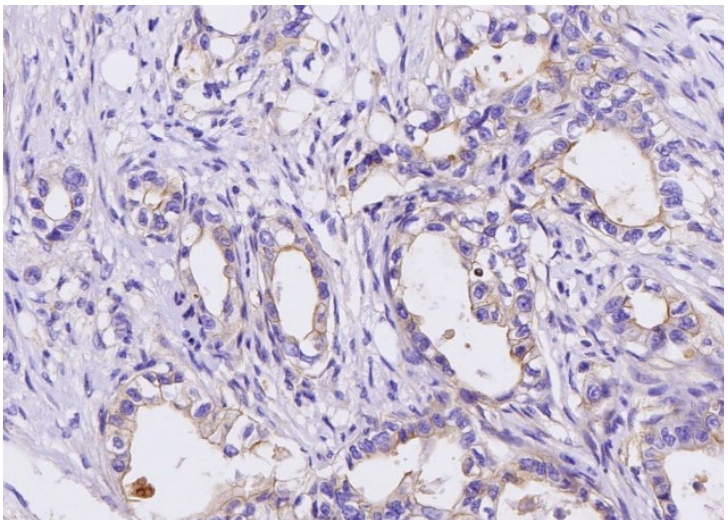
Hepatocellular carcinoma



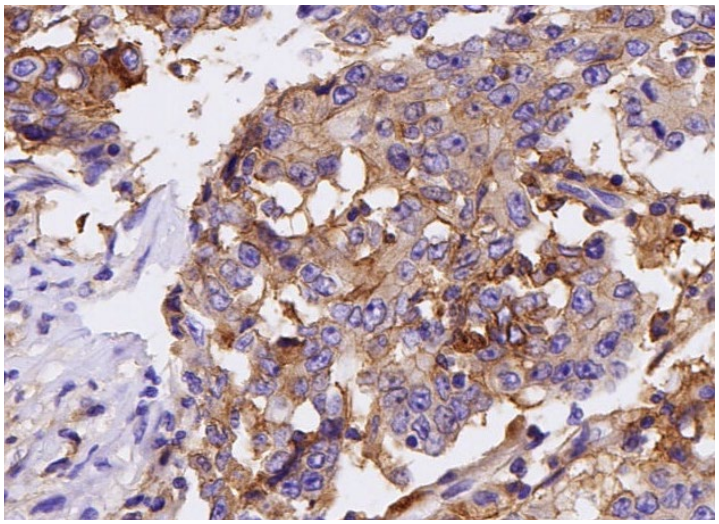
Lung adenocarcinoma



Stomach adenocarcinoma



Colon adenocarcinoma



Head and neck carcinoma

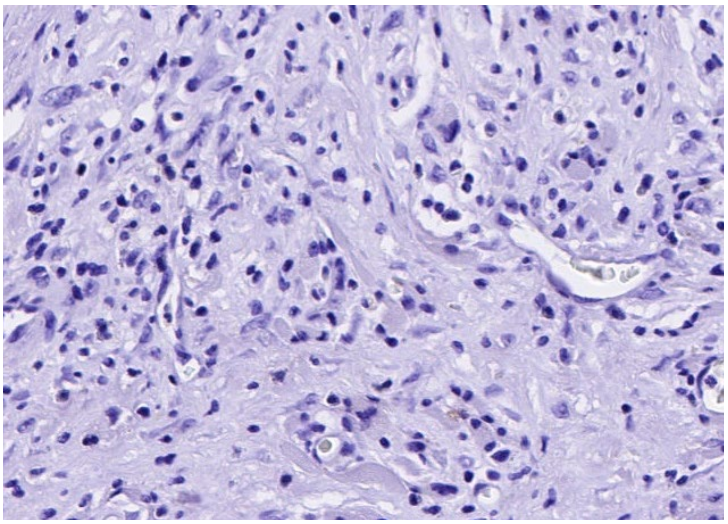
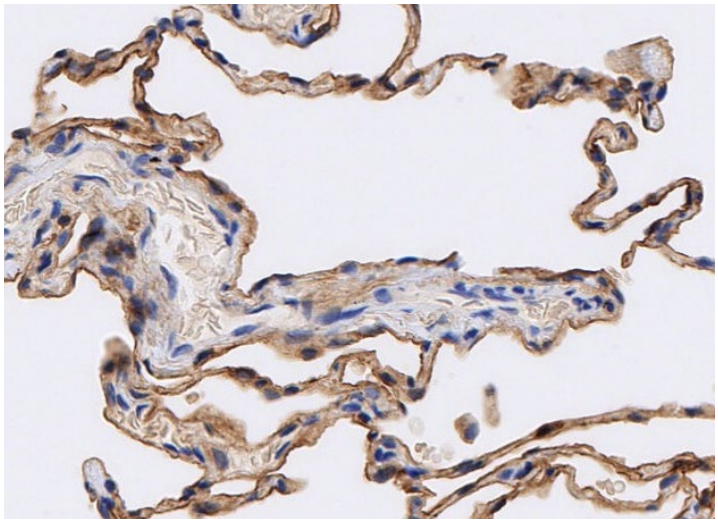


Figure 3. CD47 expression in cancer. IHC staining of multi-cancer human tissues using anti-CD47 (ab218810) (0.25ug/mL) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a BOND™ RX Research Stainer (Leica®), scanned at 20x on NanoZoomer® S360 and imaged at 20X on Aperio® ImageScope.

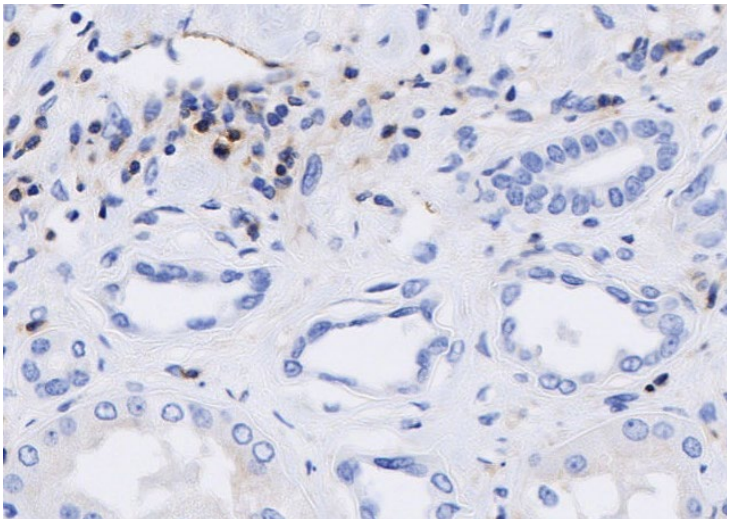
CD47 expression in multi-normal TMA (DISCOVERY ULTRA)

Below are the representative images of selected tissues from the multi-normal TMA. CD47 expression was detected in the lung, spleen, tonsil, prostate, stomach, cerebrum, and placenta, whereas low to no expression was observed in the kidney, heart pancreas and skeletal muscle tissues.

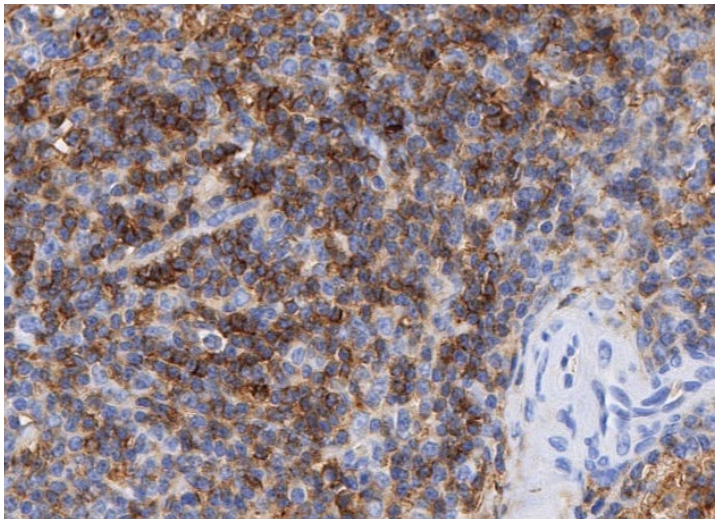
Lung



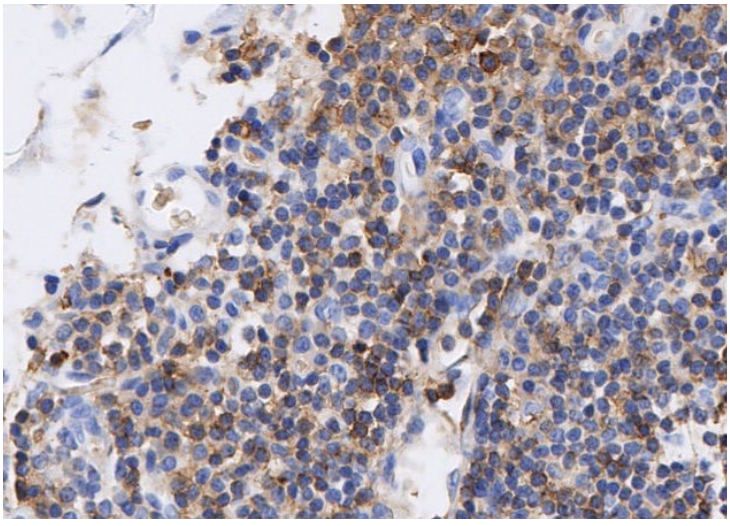
Kidney



Spleen

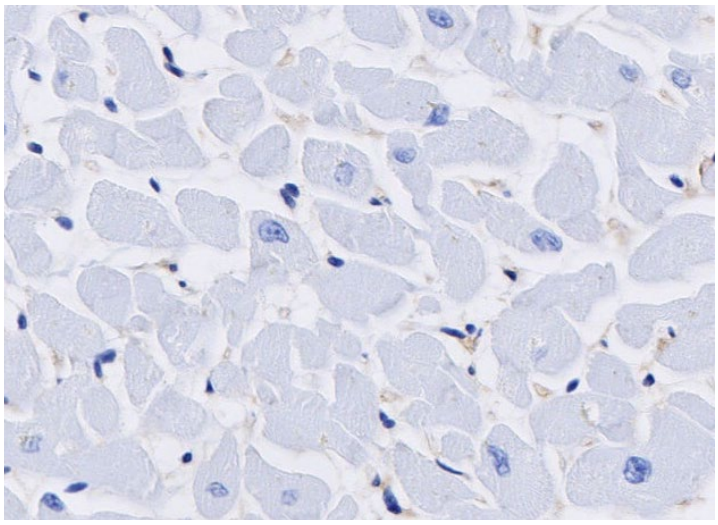


Tonsil

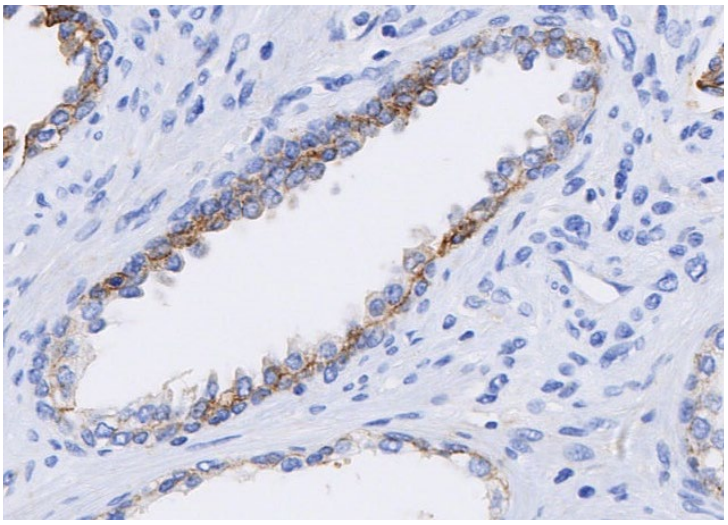


Enhanced validation data

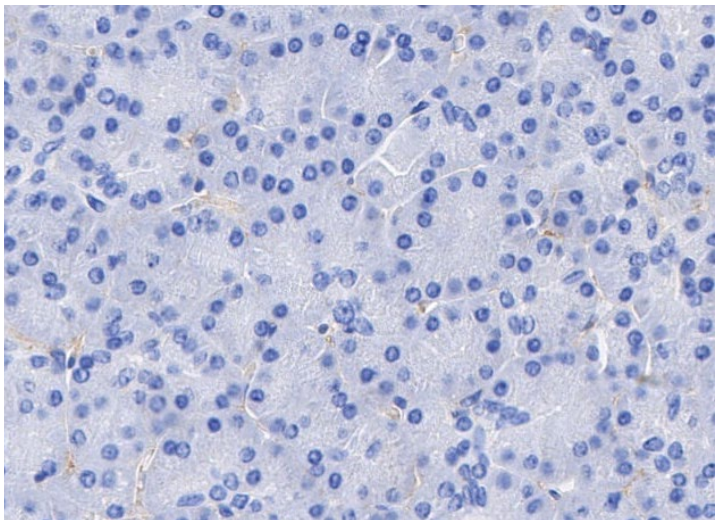
Heart



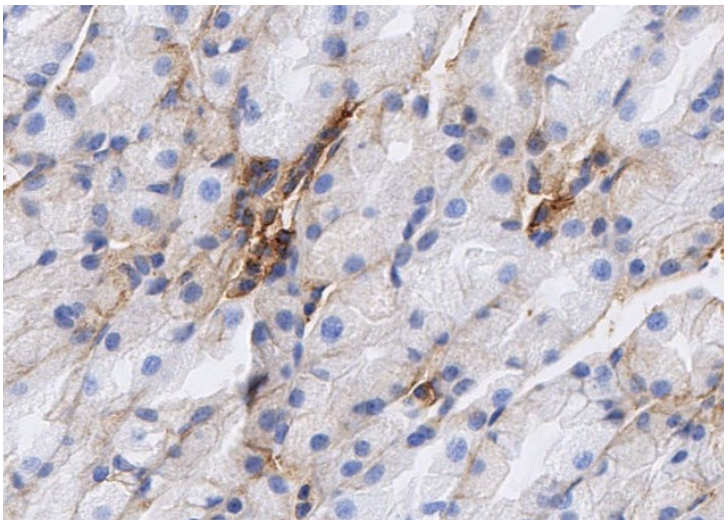
Prostate



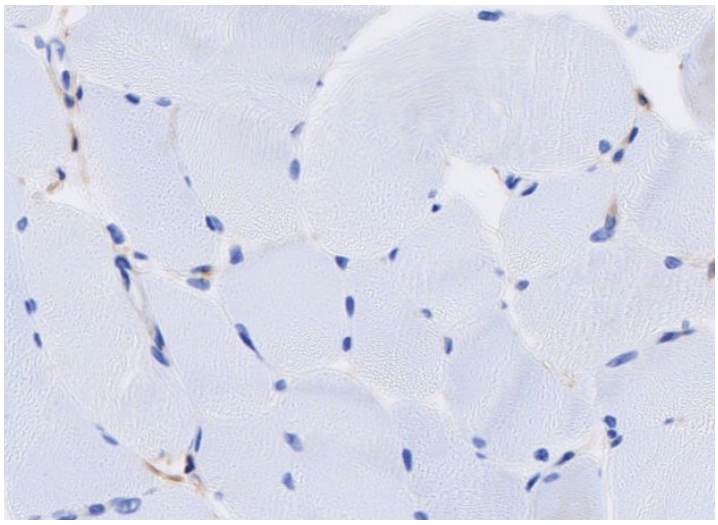
Pancreas



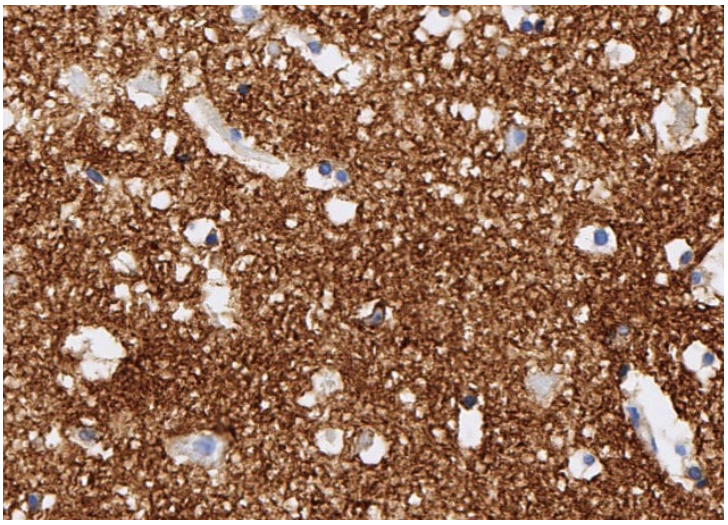
Stomach



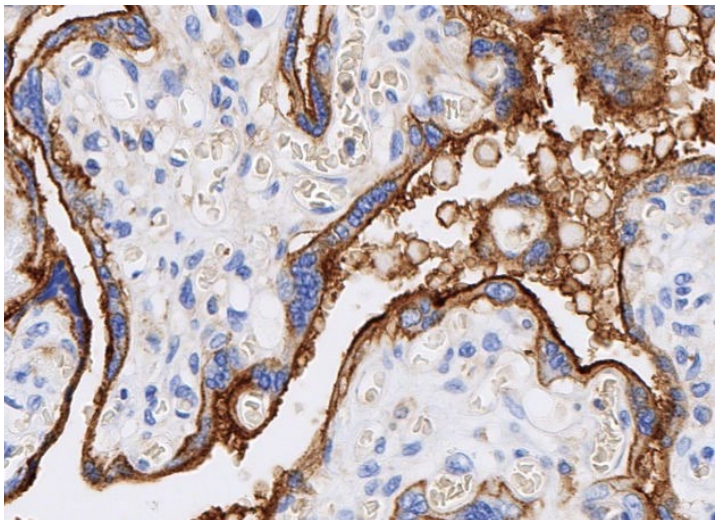
Skeletal muscle



Cerebrum



Placenta



Placenta (isotype control)

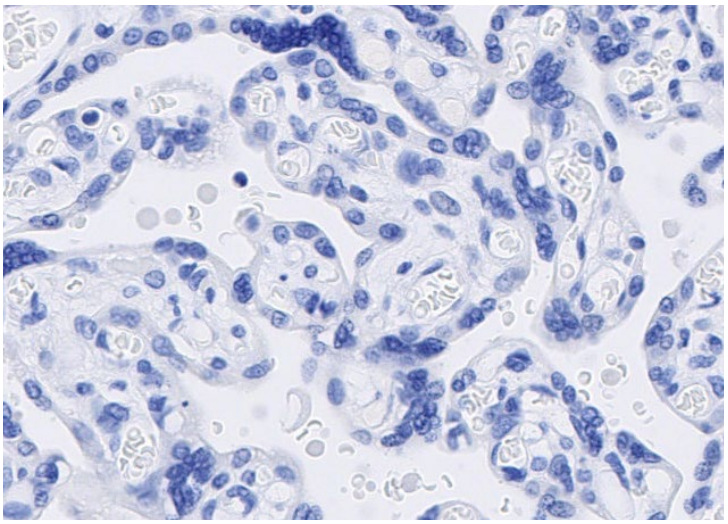


Figure 4. CD47 expression in human normal tissue. IHC staining of multi-normal human tissues using anti-CD47 (ab218810) (1ug/mL) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a DISCOVERY ULTRA system (Roche Diagnostics), scanned at 20x on NanoZoomer® S360 and imaged at 20X on Aperio® ImageScope.

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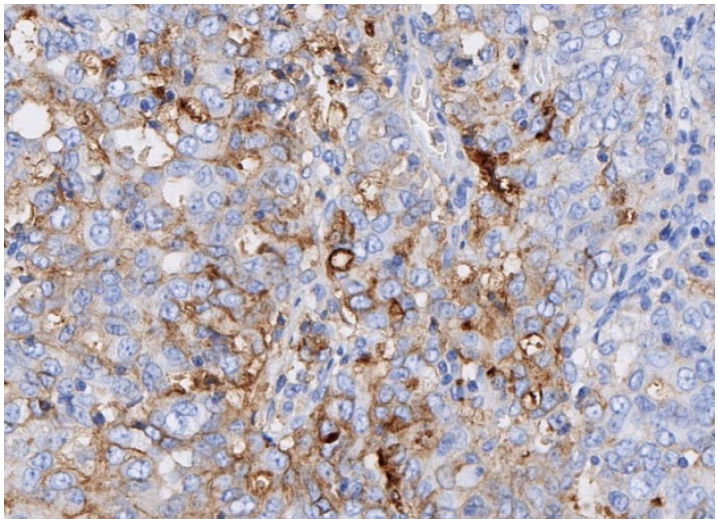
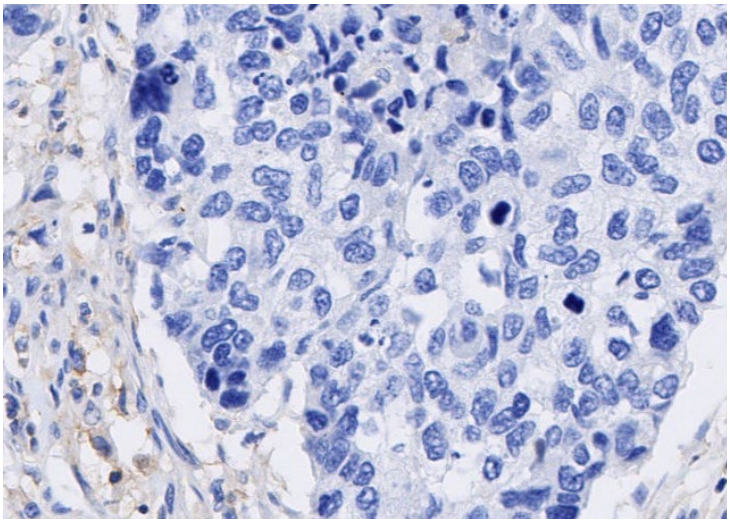
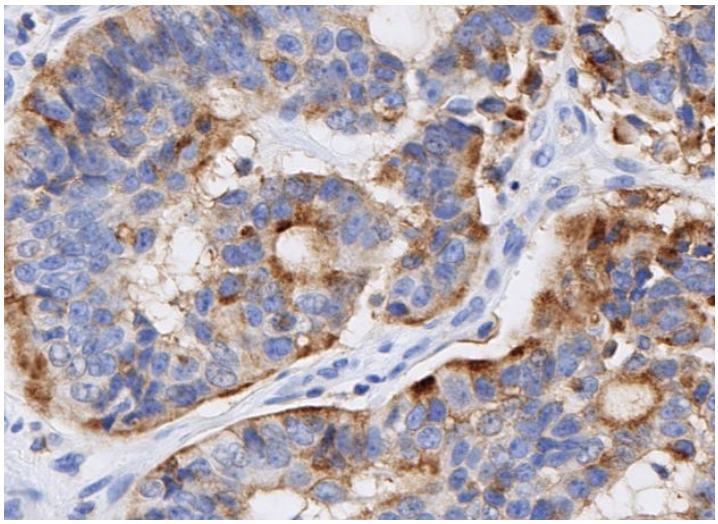
Ovarian carcinoma



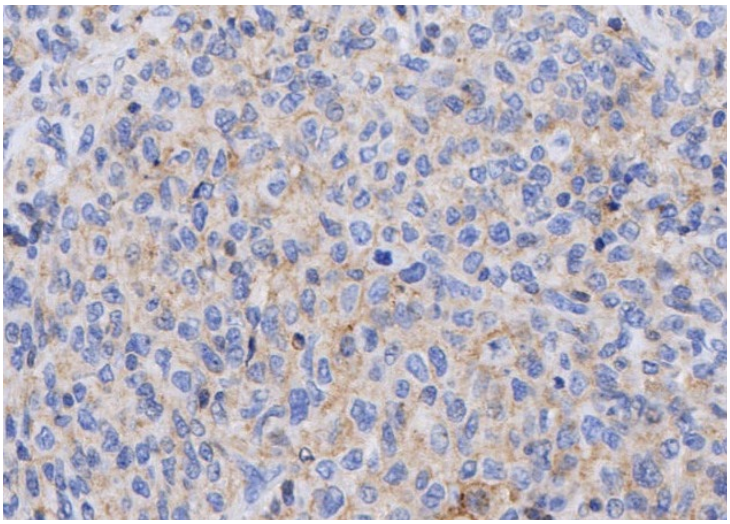
Breast ductal carcinoma



Pancreatic adenocarcinoma

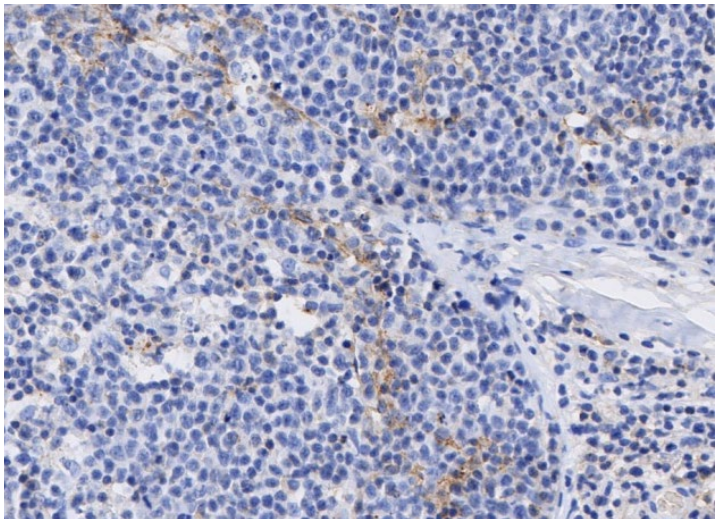


B cell lymphoma

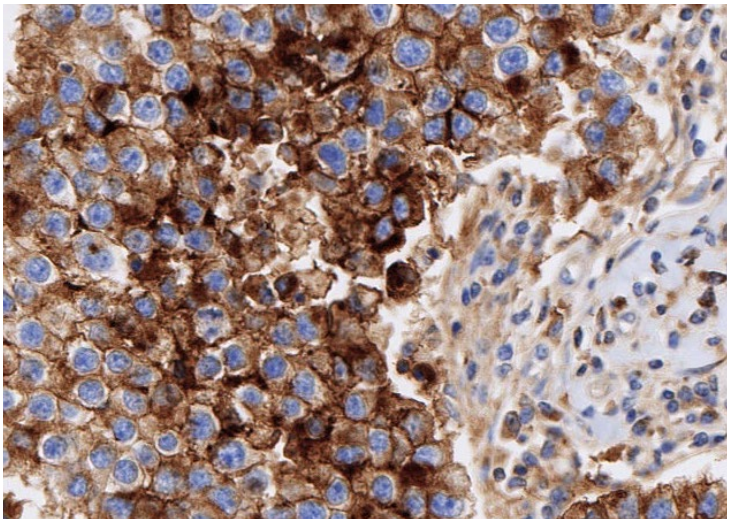


Enhanced validation data

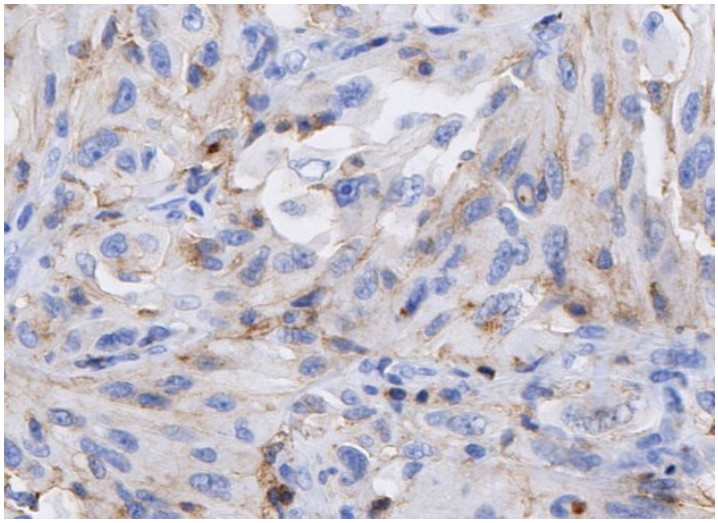
T cell lymphoma



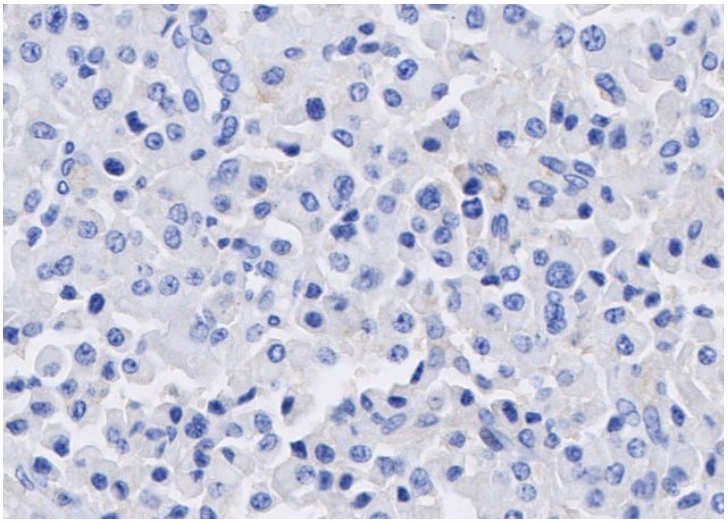
Testis seminoma



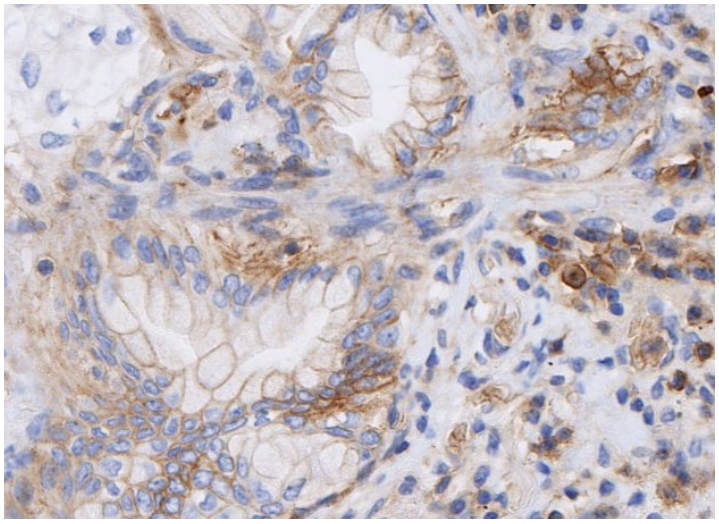
Glioblastoma



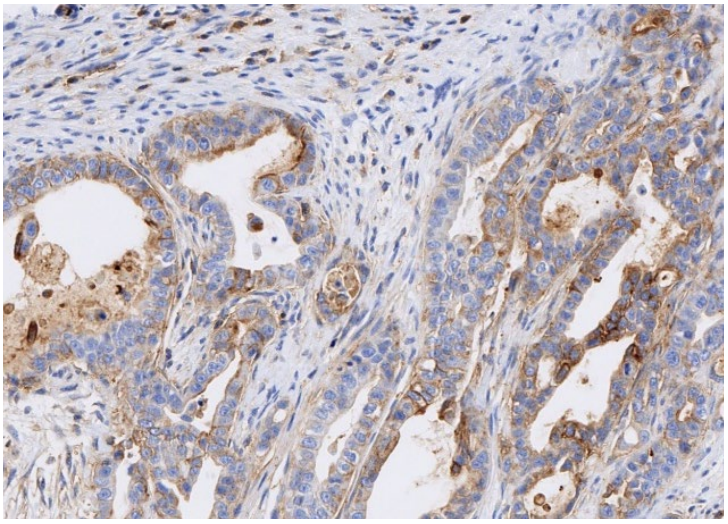
Hepatocellular carcinoma



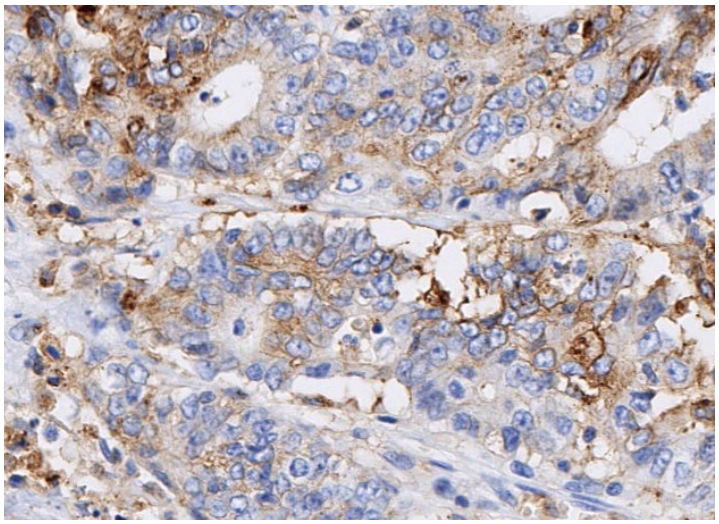
Lung adenocarcinoma



Stomach adenocarcinoma



Colon adenocarcinoma



Head and neck carcinoma

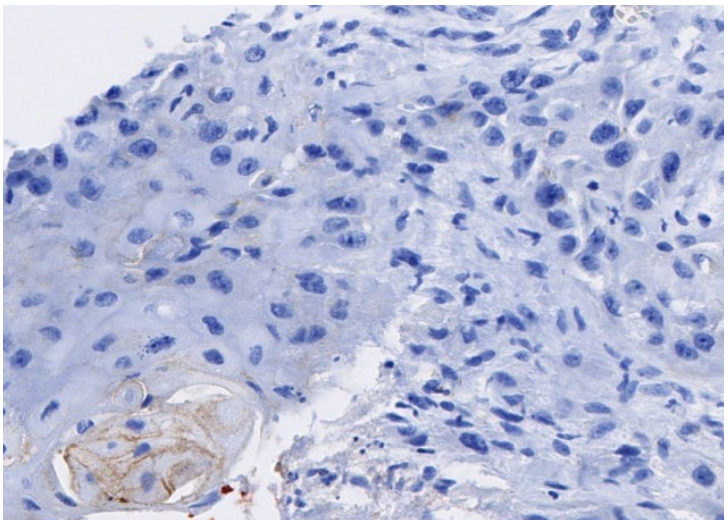
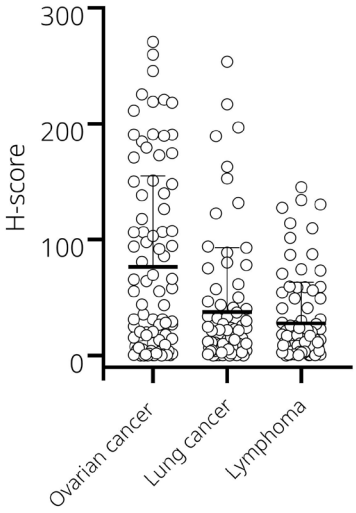


Figure 5. CD47 expression in cancer. IHC staining of multi-cancer human tissues using anti-CD47 (ab218810) (0.25ug/mL) or anti-rabbit IgG-isotype control antibody (1.0 µg/mL) (ab172730). Positive staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a DISCOVERY ULTRA system (Roche Diagnostics), scanned at 20x on NanoZoomer® S360 and imaged at 20X on Aperio® ImageScope.

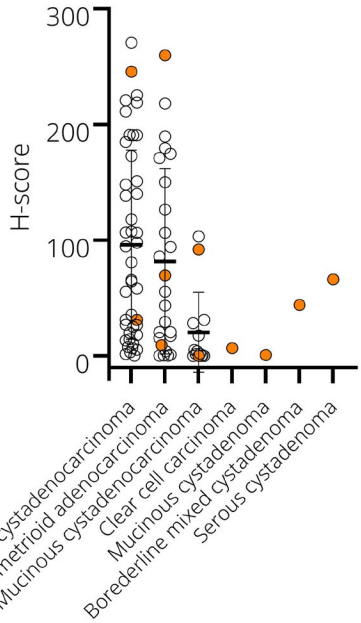
CD47 expression in cancer (BOND™ RX)

CD47 expression varied in the analyzed cancer TMAs, with ovarian cancer showing the highest cell membrane H-score and lymphoma the lowest (a). The staining intensity of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD) (b-d).

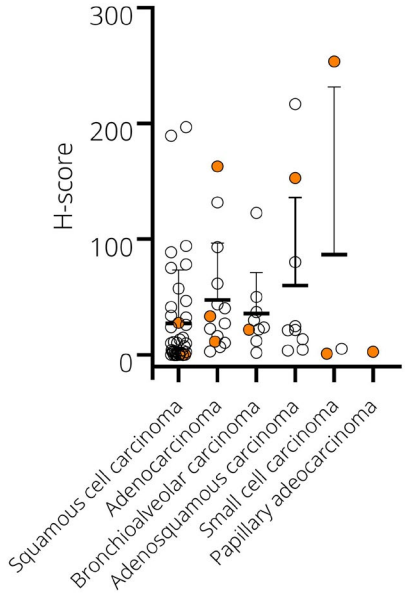
a) CD73 expression in selected cancer TMAs



b) CD47 expression in ovarian cancer



c) CD73 expression in lung cancer



d) CD73 expression in lymphoma

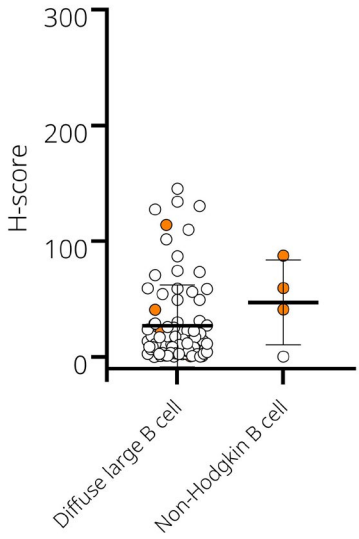
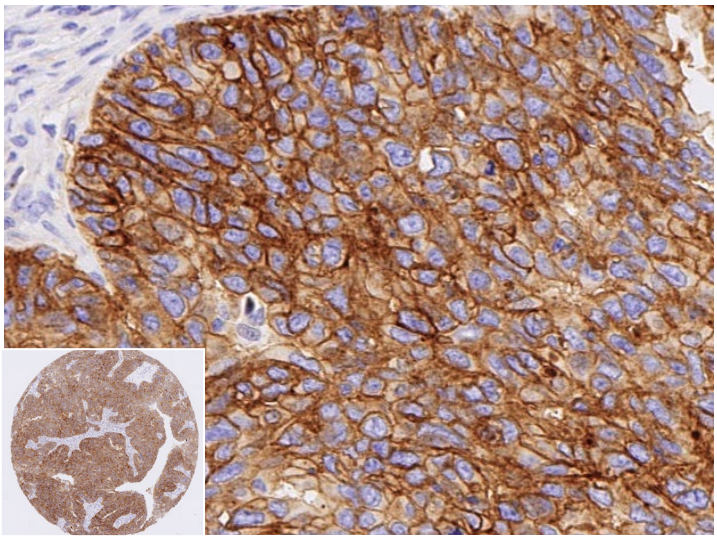


Figure 6. CD47 protein expression in a selection of cancer TMAs.
(a) The scatter plot (with SD) summarizes CD47 expression in selected cancer TMA cores (ovarian cancer (87), lung cancer (77) and lymphoma (85).
(b) H-score from tumor cell membrane staining in 87 TMA cores/cases of ovarian cancer (serous cystadenocarcinoma (44), endometrioid adenocarcinoma (25), mucinous cystadenocarcinoma (14), clear cell carcinoma (1), mucinous cystadenoma (1), borderline mixed cystadenoma (1), serous cystadenoma (1)). The IHC images corresponding to orange data points are shown in Figure 7.
(c) H-score from tumor cell membrane staining in 77 TMA cores/cases of lung cancer (squamous cell carcinoma (41), adenocarcinoma (14), bronchioalveolar carcinoma (9) adenosquamous carcinoma (9), small cell carcinoma (3), papillary adenocarcinoma (1). The IHC images corresponding to orange data points are shown in Figure 8.
(d) H-score from whole core cell membrane staining in 85 lymphoma cores/cases (diffuse large B cell (85) and non-Hodgkin B cell lymphoma (4)). The IHC images corresponding to orange data points are shown in Figure 9.

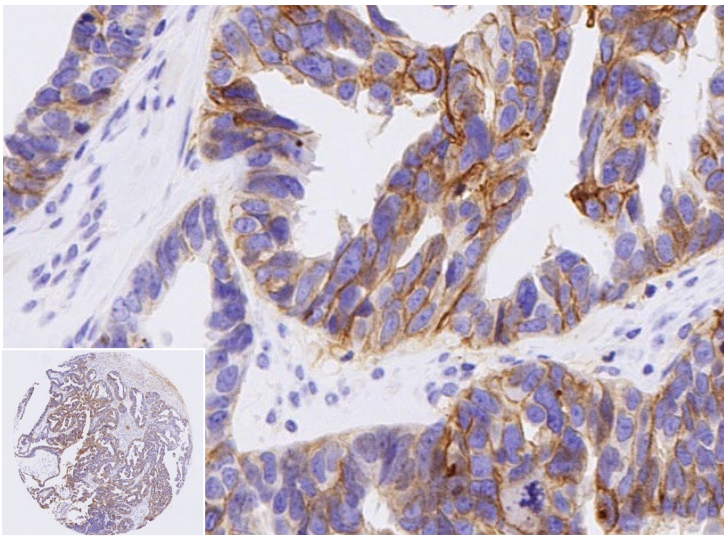
CD47 expression in ovarian cancer TMA (BOND™ RX)

Below are the representative images of the human ovarian cancer TMA showing strong to no expression of CD47 in the cell membrane.

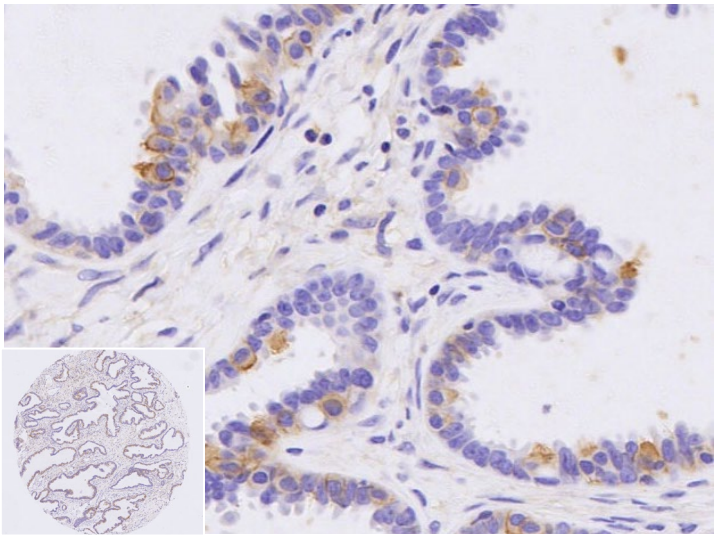
Serous cystadenocarcinoma (H-score 245.8)



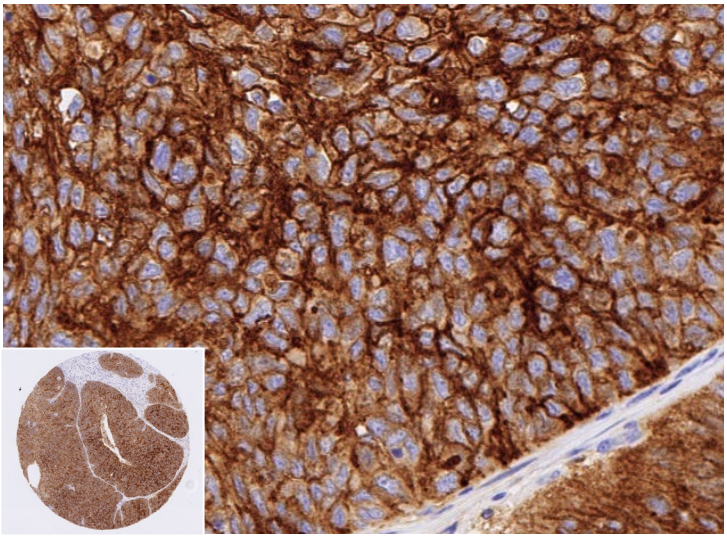
Serous cystadenocarcinoma (H-score 94.8)



Serous cystadenocarcinoma (H-score 31.4)

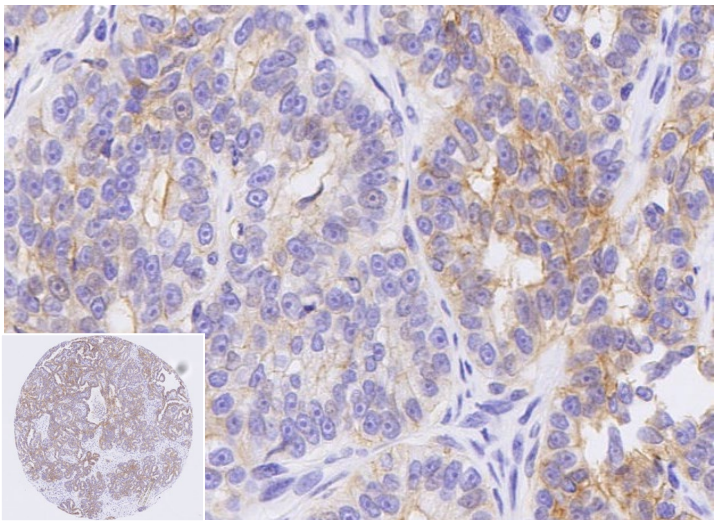


Endometrioid adenocarcinoma (H-score 259.9)

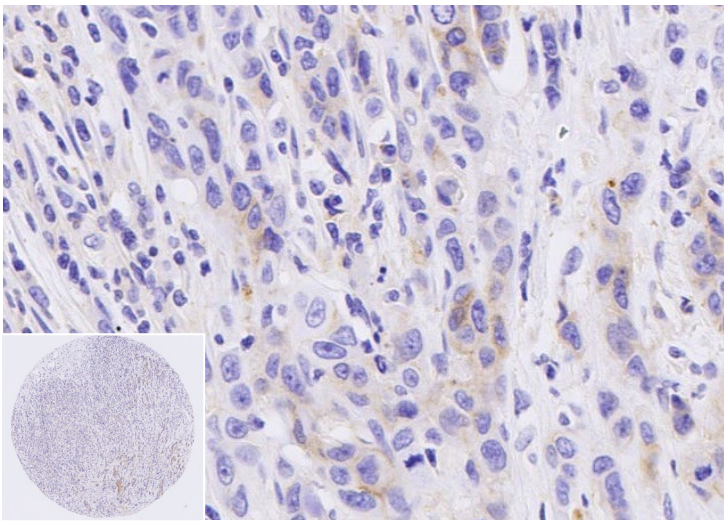


Enhanced validation data

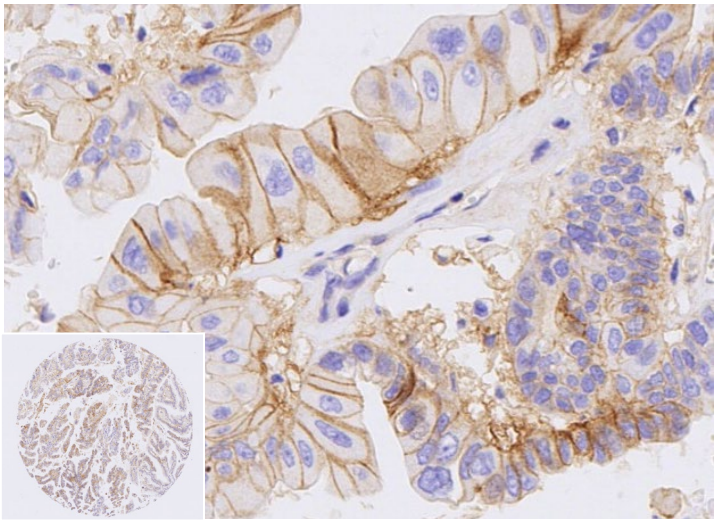
Endometrioid adenocarcinoma (H-score 69.7)



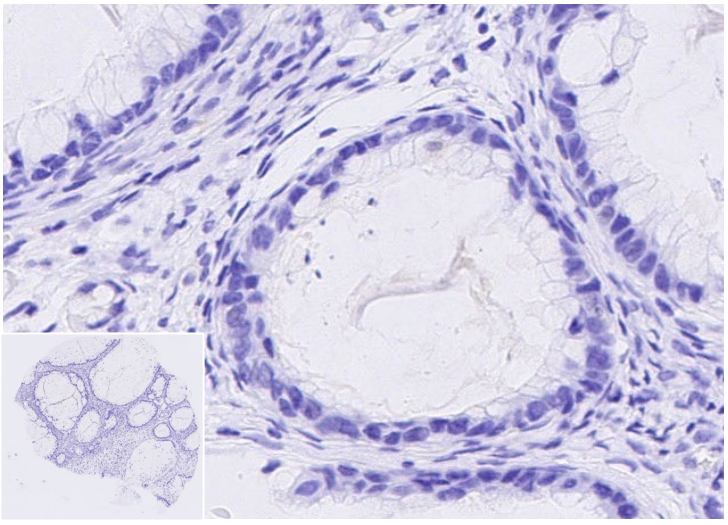
Endometrioid adenocarcinoma (H-score 9.5)



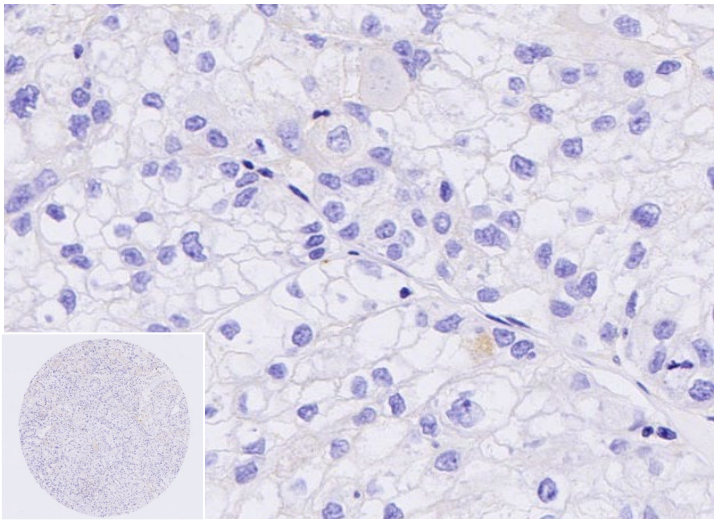
Mucinous cystadenocarcinoma (H-score 92.2)



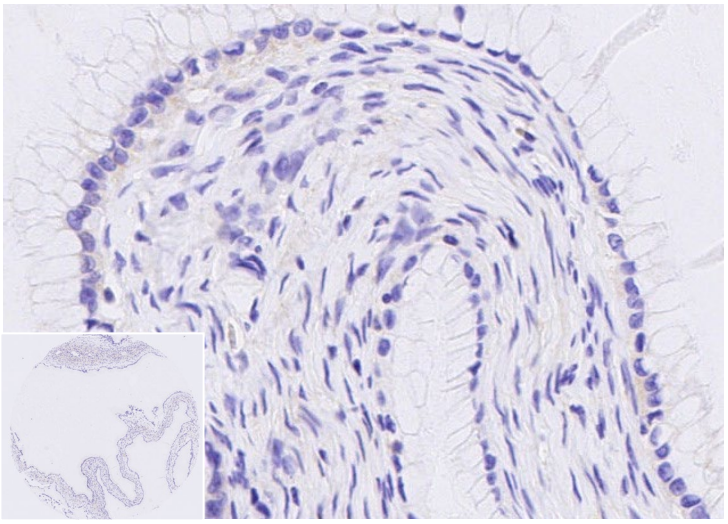
Mucinous cystadenocarcinoma (H-score 0)



Clear cell carcinoma (H-score 6.8)



Mucinous cystadenoma (H-score 0.8)



Borderline mixed cystadenoma (H-score 44.2)

Serous cystadenoma (H-score 66.3)

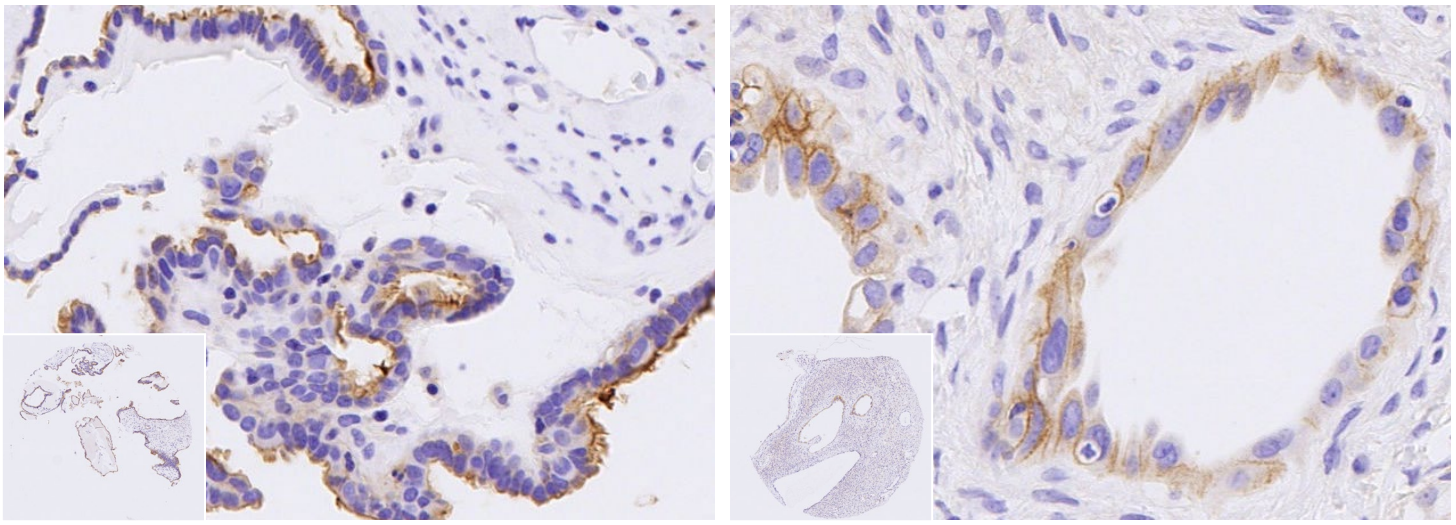
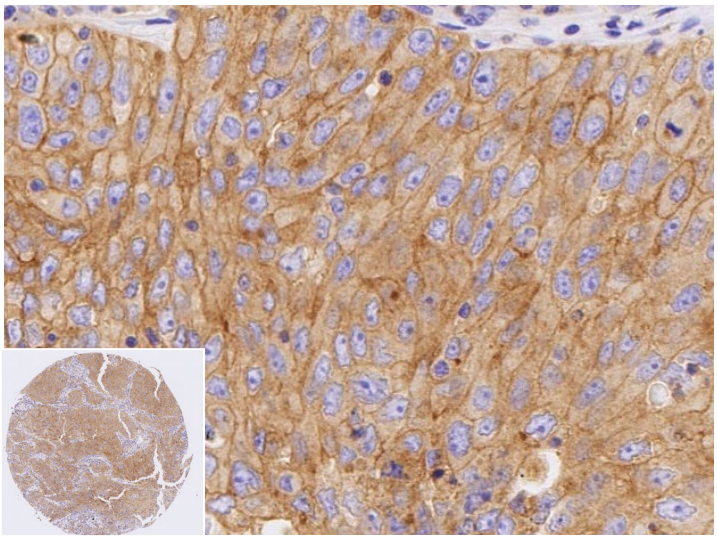


Figure 7. CD47 expression in ovarian cancer. IHC images show strong, moderate and weak staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a BOND™ RX Research Stainer (Leica®), scanned at 20x on NanoZoomer® S360 and imaged at 20x (whole core insets at 4x) on Aperio® ImageScope.

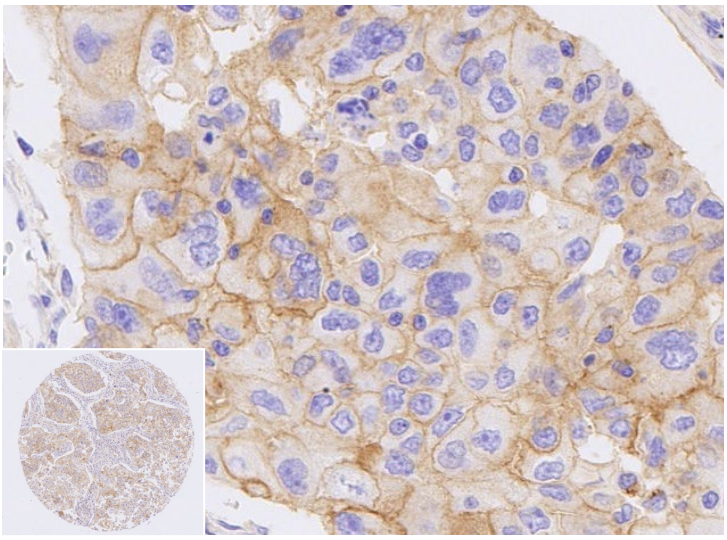
CD47 expression in lung cancer TMA (BOND™ RX)

Below are the representative images of the human lung cancer TMA showing strong to no expression of CD47 in the cell membrane.

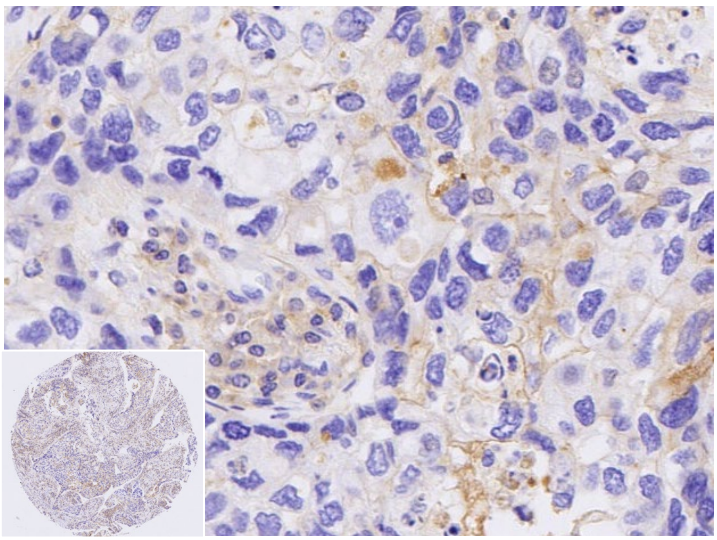
Squamous cell carcinoma (H-score 196.9)



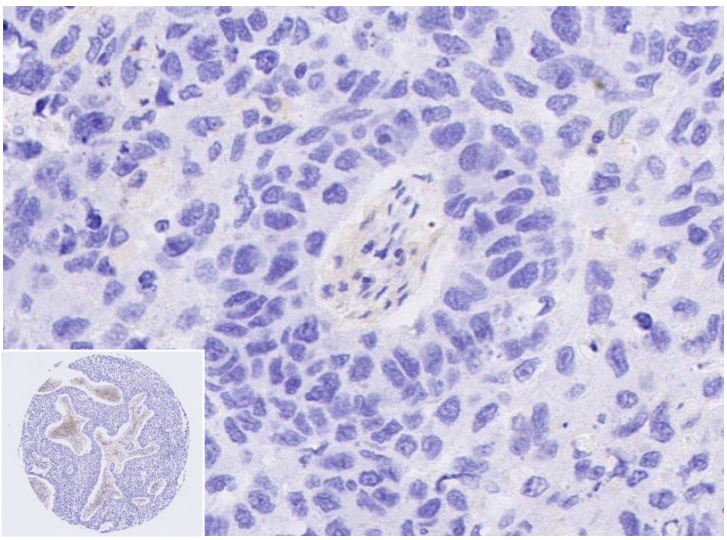
Squamous cell carcinoma (H-score 78.2)



Squamous cell carcinoma (H-score 27.8)

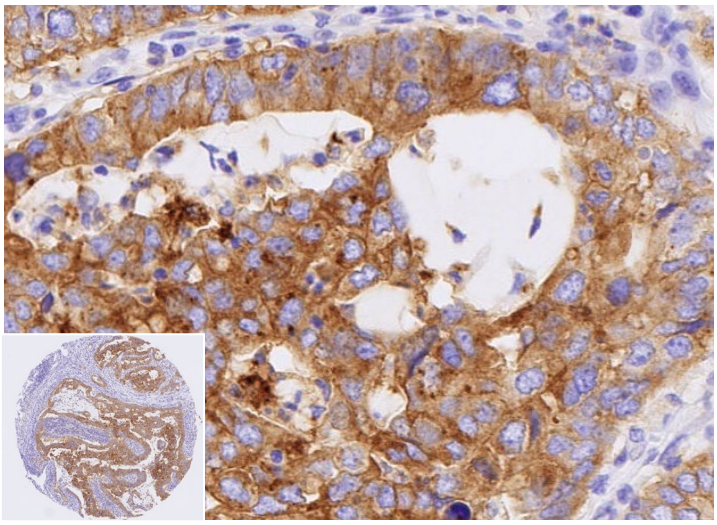


Squamous cell carcinoma (H-score 0.5)

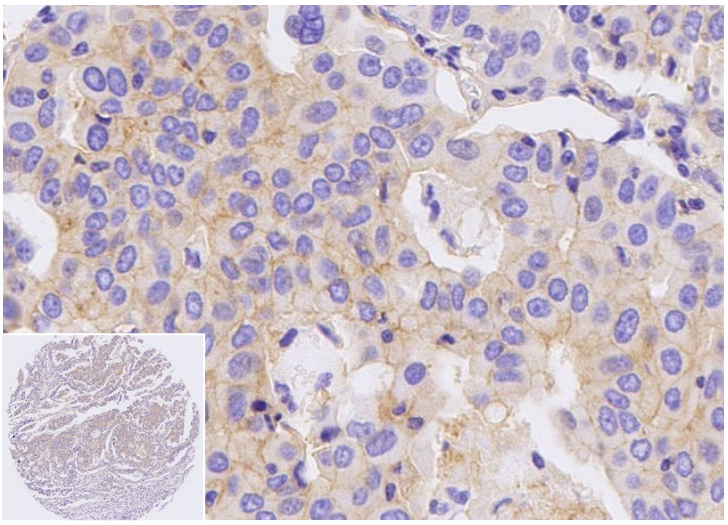


Enhanced validation data

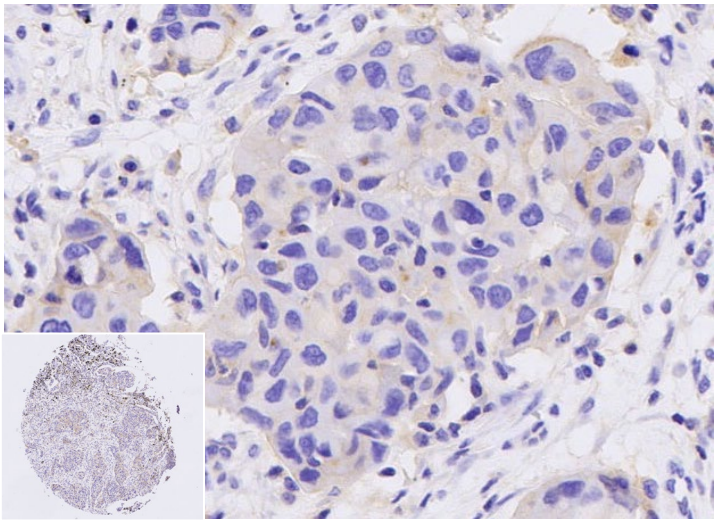
Adenocarcinoma (H-score 163.2)



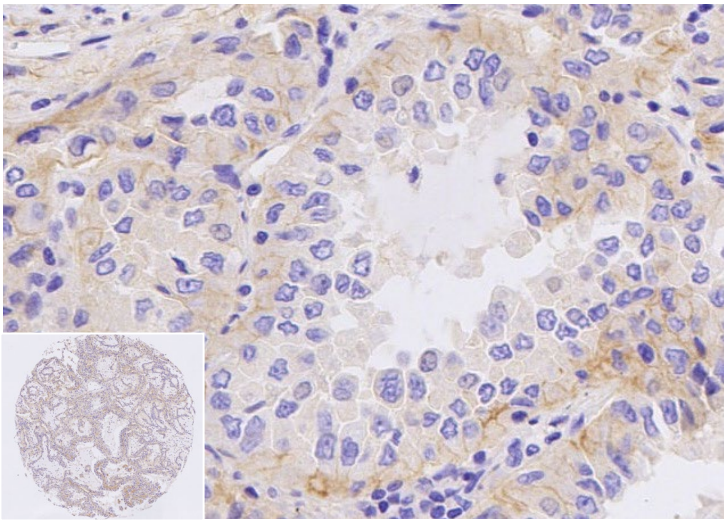
Adenocarcinoma (H-score 33.4)



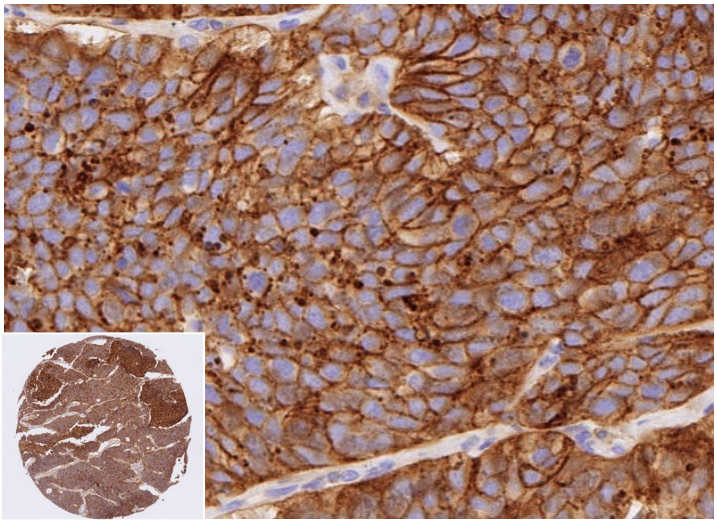
Adenocarcinoma (H-score 11.6)



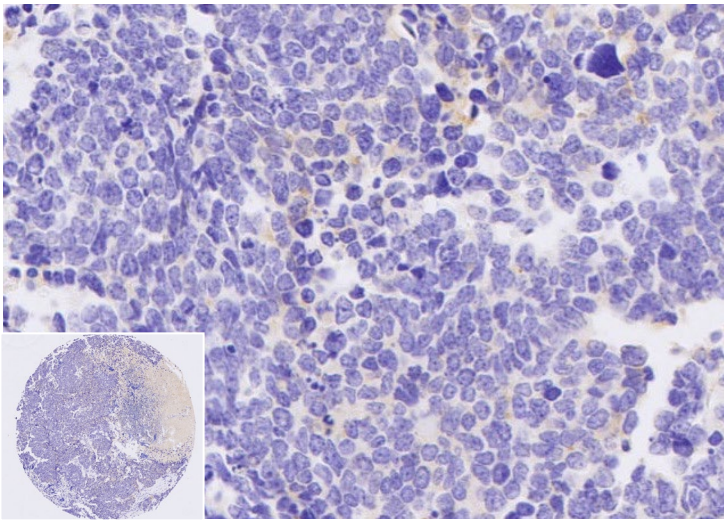
Bronchioalveolar carcinoma (H-score 22.0)



Small cell carcinoma (H-score 253.9)



Small cell carcinoma (H-score 1.2)



Papillary adeocarcinoma (H-score 2.7)

Adenosquamous carcinoma (H-score 153)

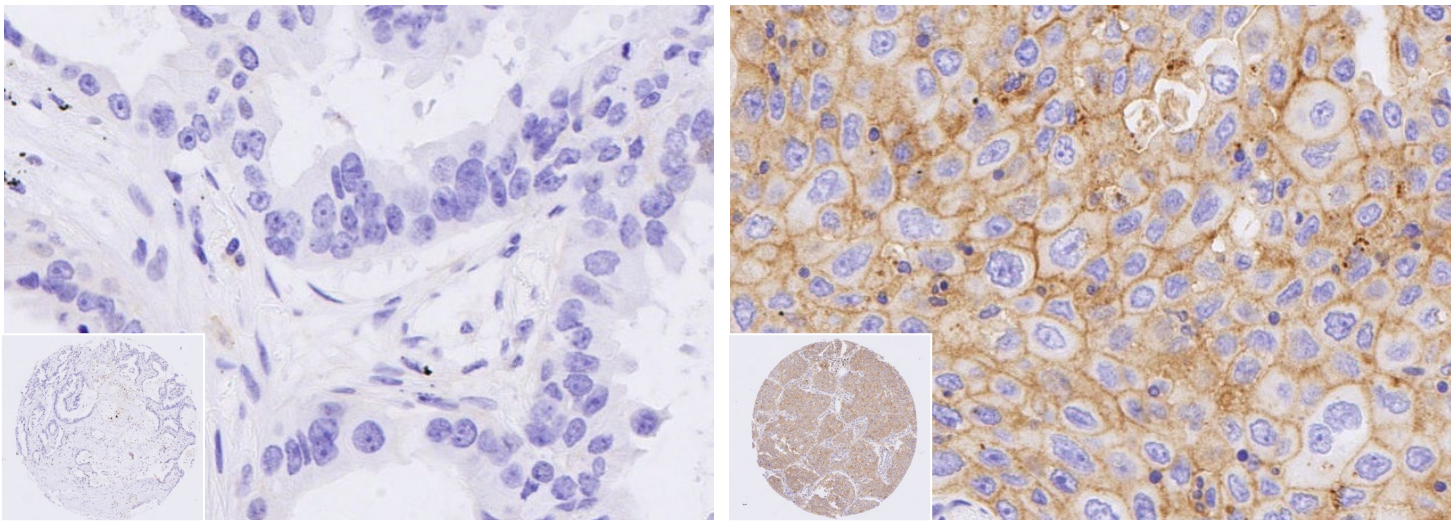
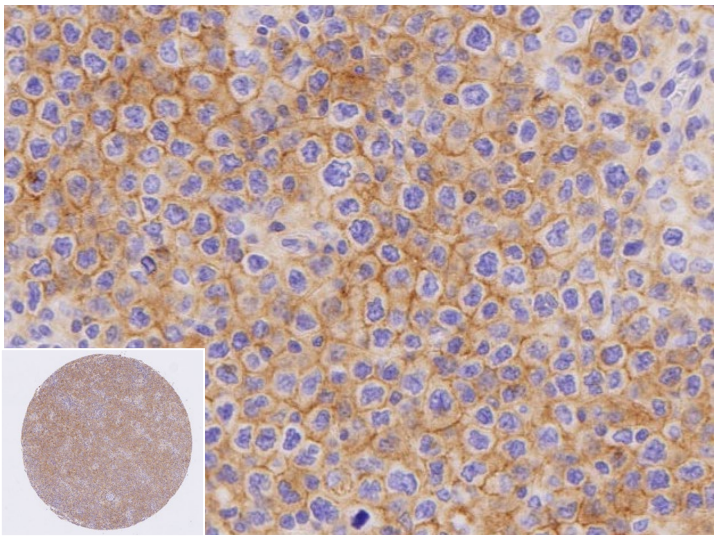


Figure 8. CD47 expression in lung cancer. IHC images show strong, moderate and weak staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a BOND™ RX Research Stainer (Leica®), scanned at 20x on NanoZoomer® S360 and imaged at 20x (whole core insets at 4x) on Aperio® ImageScope.

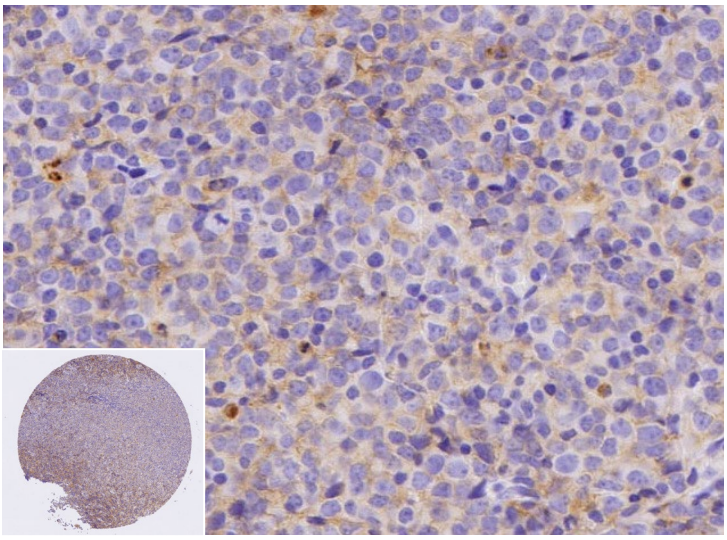
CD47 expression in lymphoma TMA (BOND™ RX)

Below are the representative images of the human lymphoma TMA showing strong to no expression of CD47 in the cell membrane.

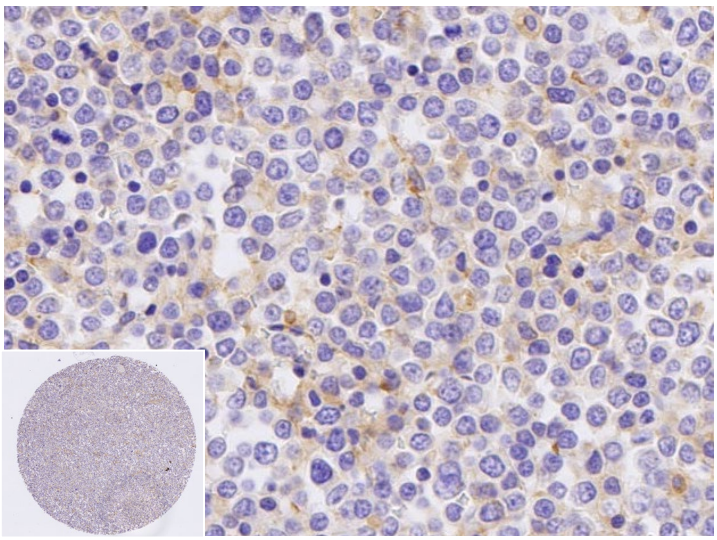
Diffuse large B cell lymphoma (H-score 114.3)



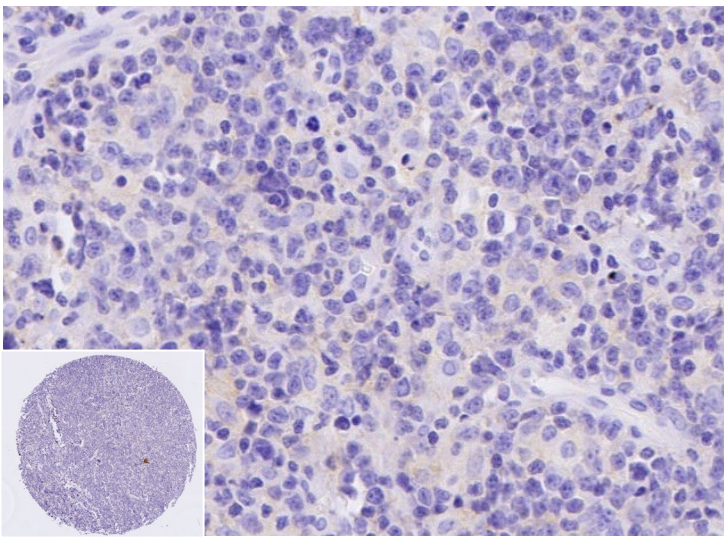
Diffuse large B cell lymphoma (H-score 40.9)



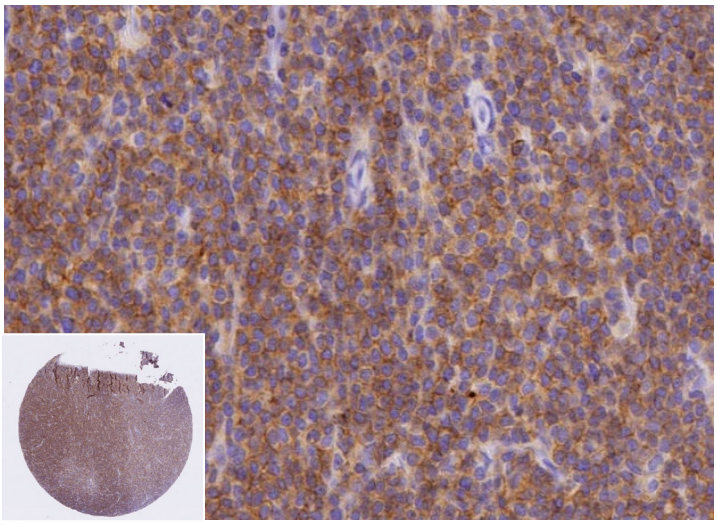
Diffuse large B cell lymphoma (H-score 21.5)



Diffuse large B cell lymphoma (H-score 0.7)



Non-Hodgkin B cell lymphoma (H-score 87.6)



Non-Hodgkin B cell lymphoma (H-score 41.2)

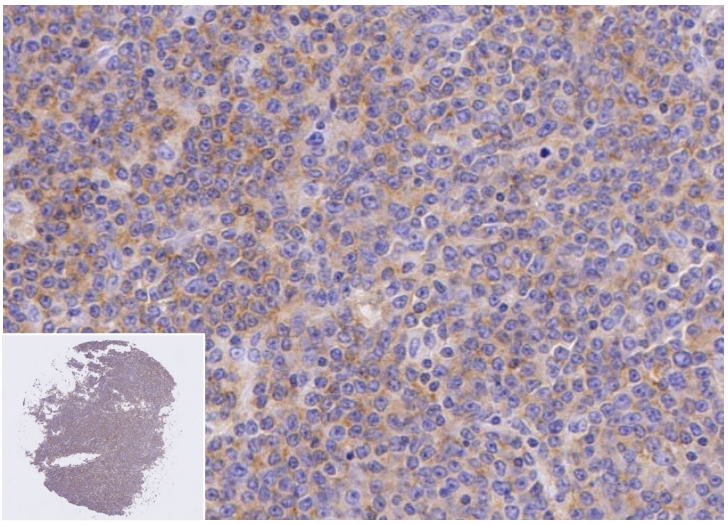


Figure 9. CD47 expression in lymphoma cancer. IHC images show strong, moderate and weak staining in brown; nuclear hematoxylin counterstain in blue. Slides were stained using a BOND™ RX Research Stainer (Leica®), scanned at 20x on NanoZoomer® S360 and imaged at 20x (whole core insets at 4x) on Aperio® ImageScope.

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