Enhanced validation data

Anti-TROP2 recombinant antibody – ab214488

AB0332 2024/05 A

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Enhanced validation of Anti-TROP2 recombinant antibody [EPR20043] – ab214488

Enhanced validation designed for your needs

We understand the challenge of finding the right antibody clone – highly specific and sensitive to your intended target – at early selection stages of your development program. To de-risk this clone selection process for you, we generated enhanced validation data for our best recombinant antibody clones to some of the most promising targets.

Our enhanced validation gives you an extra level of confidence in an antibody clone

— Provides additional data on the specificity and sensitivity of our recombinant antibodies in immunohistochemistry (IHC) and other relevant techniques

— Carried out in a custom manner, specific both to the target and the relevant research & clinical settings

— Builds upon our high-quality standard validation

Our framework for enhanced validation

— Our enhanced validation focuses on generating detailed IHC expression profiles for promising 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- TROP2 recombinant antibody [EPR20043] – ab214488 in IHC in selected tissues, cancer TMAs and knock-out cell lines using a BOND™ RX Research Stainer (Leica®) (results in Figures 1-8).

— An assay was also developed using the DISCOVERY ULTRA system (Roche diagnostics) (results in Figure 9).

Target overview

HGNC symbol
TACSTD2

Approved name
Tumor-associated calcium signal transducer 2

Alternative names
Cell surface glycoprotein Trop-2
Membrane component chromosome 1 surface marker 1
Pancreatic carcinoma marker protein GA733-1

Chromosomal location
1p32.1

Function

- Trophoblast cell surface antigen 2 (TROP2) is a cell surface receptor that transduces intracellular calcium signaling to promote tumor proliferation and metastasis[1-6].
- Identified in trophoblasts which are invasive cells of the placenta that form the outer layer of the blastocyst[7,8].
- Epithelial tumors frequently express TROP2. A tumor-type-dependent association exists between its abnormal expression and cancer progression[8].

Tissue specificity

- Expressed by normal epithelial cells of tonsil, skin, lungs, salivary glands, breast, endometrium, urothelium and prostate[8].
- High TROP2 expression in squamous cell carcinomas of various origins and various categories of urothelial, breast, prostate, pancreatic, and ovarian cancers[1,8].

Cellular localization

- Cell membrane.

Target information above taken in part from: UniProt accession P09758
The UniProt Consortium
The Universal Protein Resource (UniProt) in 2010
Nucleic Acids Res. 38:D142-D148 (2010)

Materials and methods

Tissue microarray (TMA)	Cores	Cases	Normal/ Benign cases	Cancer cases	Source (#catalog number)
Multi-normal	37	20	20	0	Abcam
Breast cancer (triple negative)	96	48	0	48	Pantomics (#BRC964)
Breast cancer	150	70	5	70	Pantomics (#BRC1502)
Lung cancer	102	102	5	97	Pantomics (#LUC1021)
Ovary cancer	102	102	5	97	Pantomics (#OVC1021)
EMC1021	102	102	5	97	Pantomics (#EMC1021)

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

Step	Reagents	Method
Deparaffinization	EZ Prep	Standard
Cell conditioning	ULTRA Cell Conditioning Solution (ULTRA CC1)	32 min, 100 °C
Pre-primary peroxidase inhibitor	OptiView Peroxidase Inhibitor	4 min
Primary antibody	Recombinant anti-TROP2 rabbit monoclonal [EPR20043] antibody – ab214488 diluted in Bond™ primary antibody diluent (AR9352) to final concentration of 0.05 µg/mL	32 minutes, 36 °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.

Step	Reagents	Pre-programmed protocol
Dewax	Bond™ dewax solution (AR922), alcohol, BOND wash solution (AR9590)	Dewax
Antigen retrieval	Bond™ epitope retrieval ER1 solution (AR9961)	HIER with ER1 (pH 5.9–6.1), 20 minutes, 100°C

Table 3a. IHC prestaining protocol on BOND™ RX Research Stainer (Leica®).

Step	Reagents	Number of washes	Time (minutes)
Peroxide block	3-4% (v/v) Hydrogen peroxide	-	5
Wash	Bond™ wash solution	3x	0
Primary antibody	Recombinant anti-TROP2 rabbit monoclonal [EPR20043] antibody – ab214488 diluted in Bond™ primary antibody diluent (AR9352) to final concentration of 0.3 µg/mL	-	15
Wash	Bond™ wash solution	3x	0
Secondary antibody	Bond™ polymer refine detection (DS9800)	-	8
Wash	Bond™ wash solution	2x	4
	Deionized water	1x	0
Visualization	Mixed DAB refine (DS9800)	1x	0
	Mixed DAB refine (DS9800)	-	10
Wash	Deionized water	3x	0
Counterstain	Hematoxylin (DS9800)	-	5
Wash	Deionized water	1x	0
	Bond™ wash solution	1x	0
	Deionized water	1x	0

Table 3b. 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 anaylsis of TROP2 expression was performed using the artificial intelligence (AI) driven digital image analysis software Visiopharm® (Version: 2023.09). TMA slides were de-arrayed and segmented into tumor and non-tumor using a trained AI model with DeepLabv3+ architecture. The segmentation performance was assessed by eye and adjusted where necessary.

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

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

Table 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 = [(0 x % 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.

TROP2 expression in FFPE cell pellets (BOND™ RX)

Below are the representative images of TROP2 expression in TACSTD2 wild-type and knock-out FFPE MCF7 (human breast adenocarcinoma cancer) cells (ab286330). TROP2 was detected in the wild-type cell line and absent in the knock-out cell line.

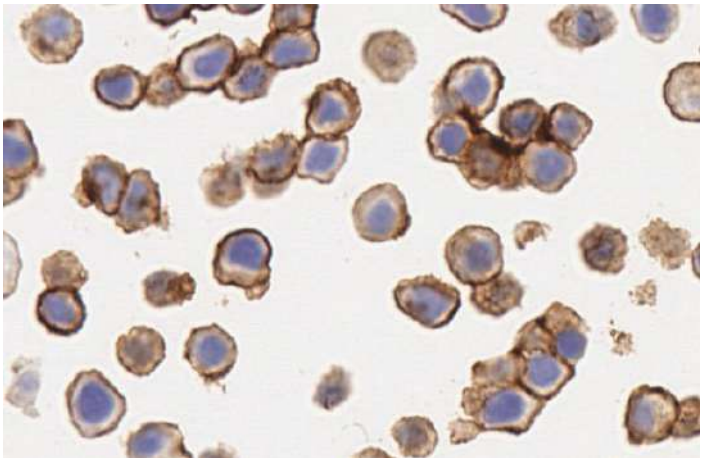
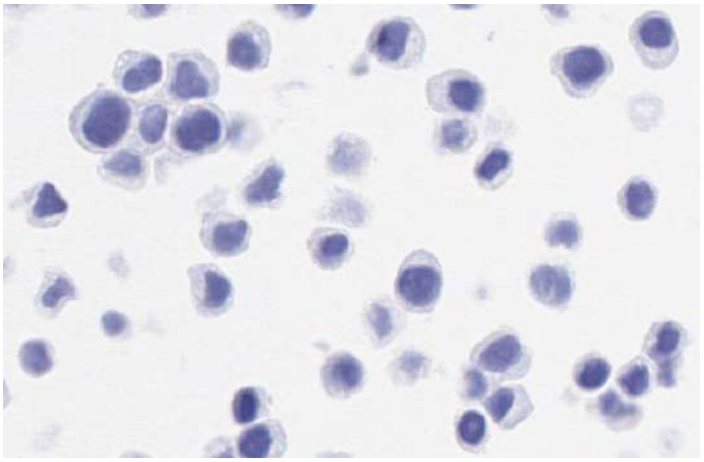
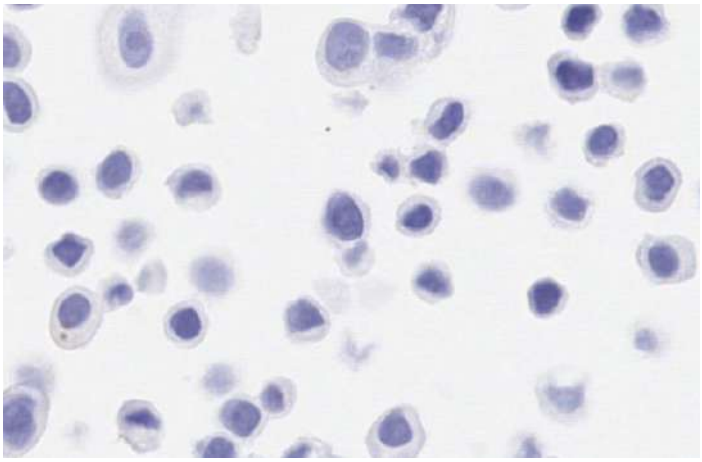
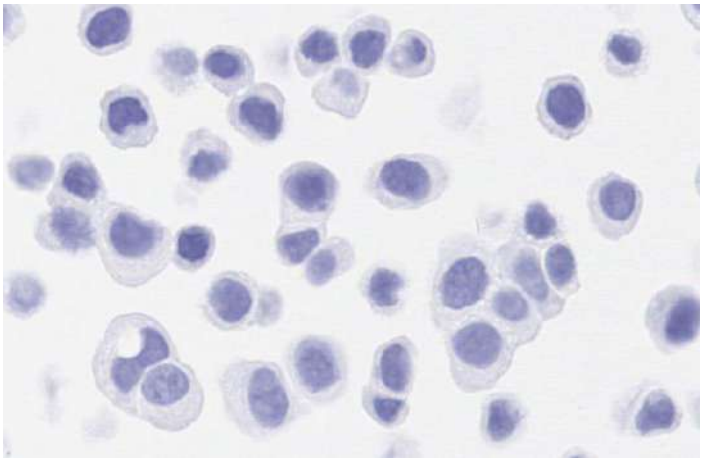
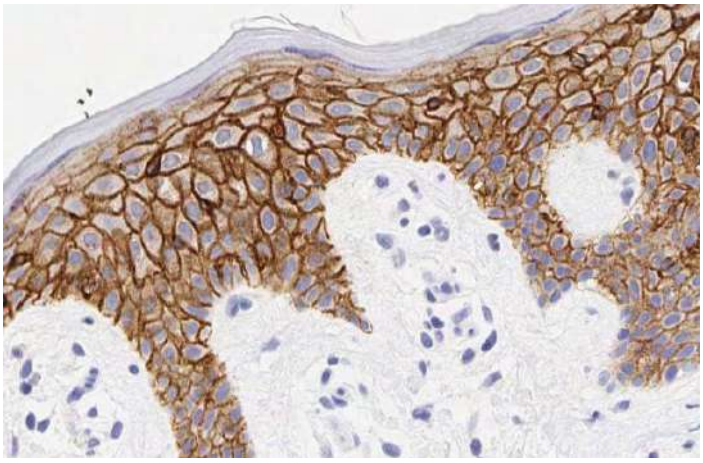
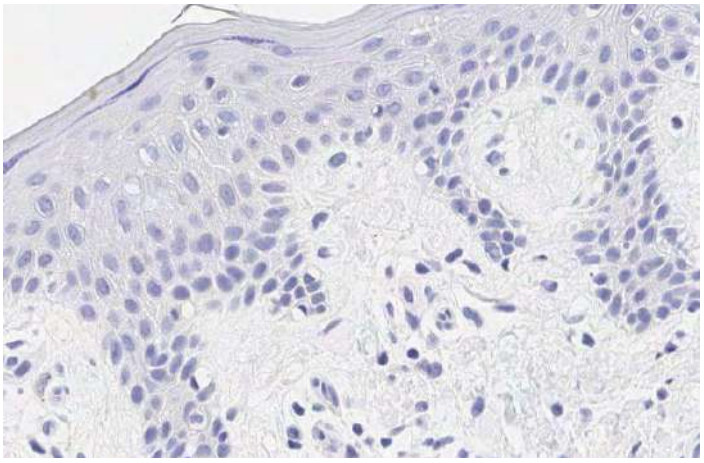
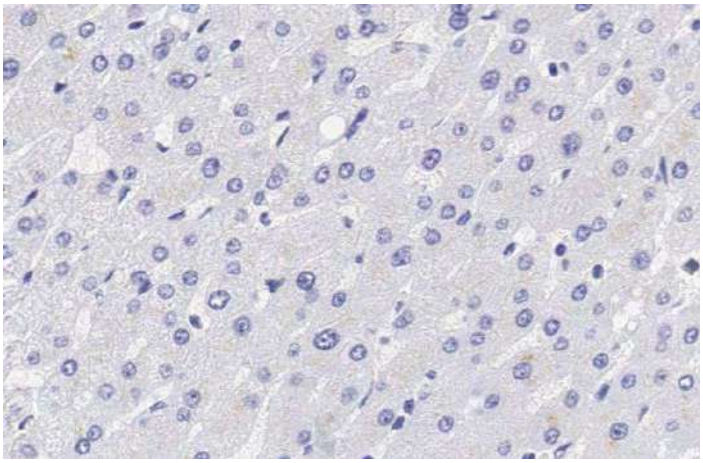
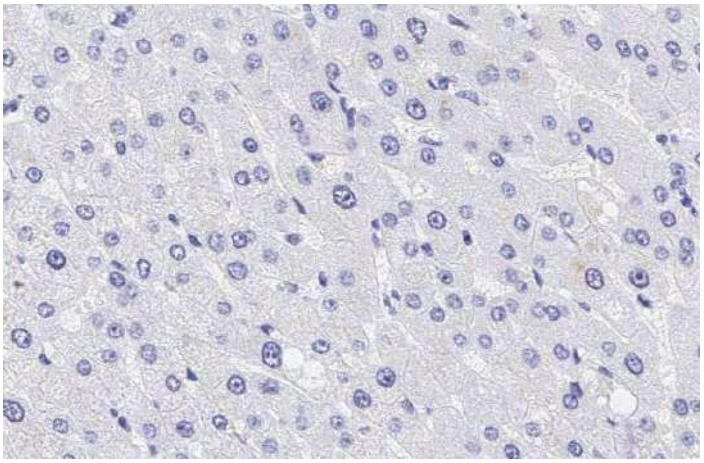
MCF7 $^{TACSTD2\ +/+}$	**MCF7** $^{TACSTD2\ -/-}$
TROP2	
	
Isotype control	
	

Figure 1. TROP2 expression in TACSTD2 wild-type and knock-out MCF cell lines.
Immunohistochemical analysis of FFPE MCF7$^{TACSTD2\ +/+}$ and MCF7$^{TACSTD2\ -/-}$ (human breast adenocarcinoma cancer) cell lines. IHC staining of FFPE cell pellets using anti-TROP2 (ab214488) or rabbit IgG–isotype control antibody (ab172730) at a final concentration of 0.3ug/ml. Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 40x on Aperio® AT2 and imaged at 40x on Aperio® ImageScope.

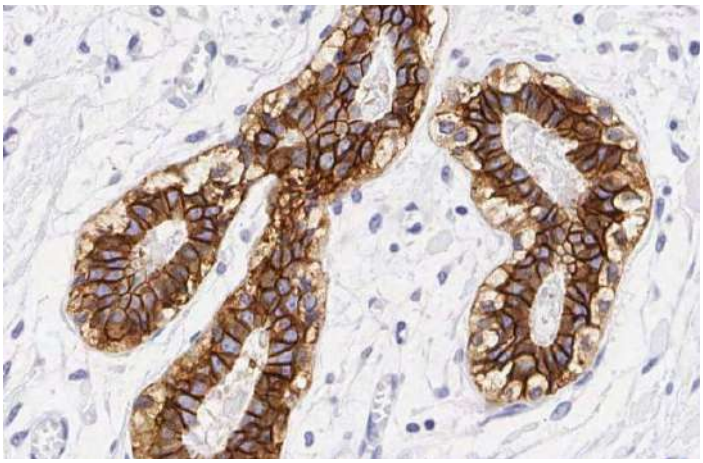
TROP2 expression in normal human tissues (BOND™ RX)

Below are the representative images of selected human normal tissues. TROP2 expression was detected in skin, breast, endometrium and lung. Expression was absent from the liver and ovary.

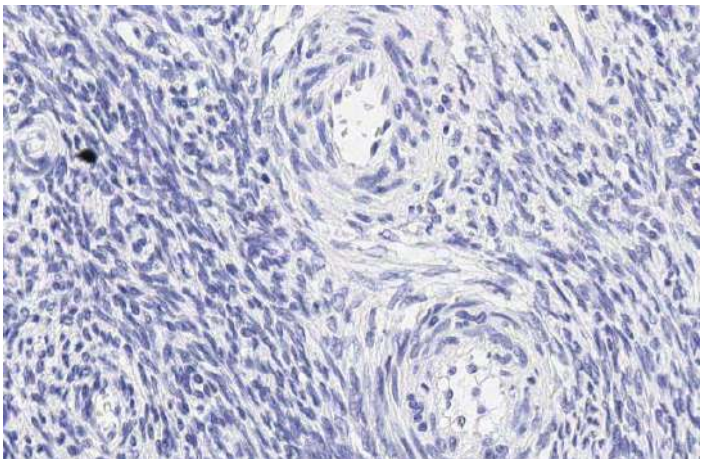
TROP2	Isotype Control
Skin	
	
Liver	
	

TROP2

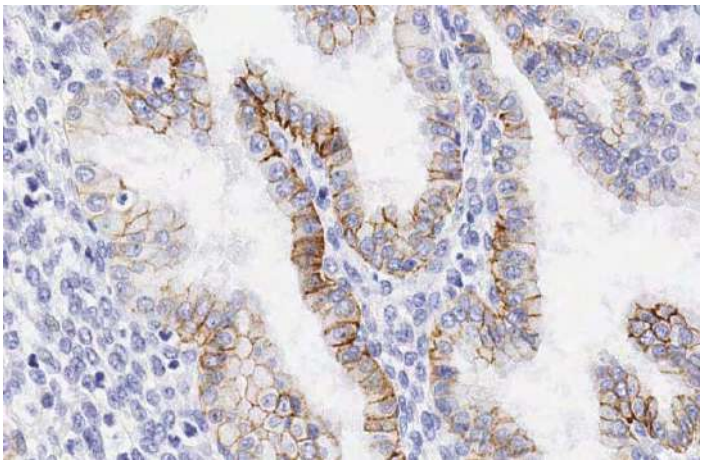
Breast



Ovary



Endometrium



Lung

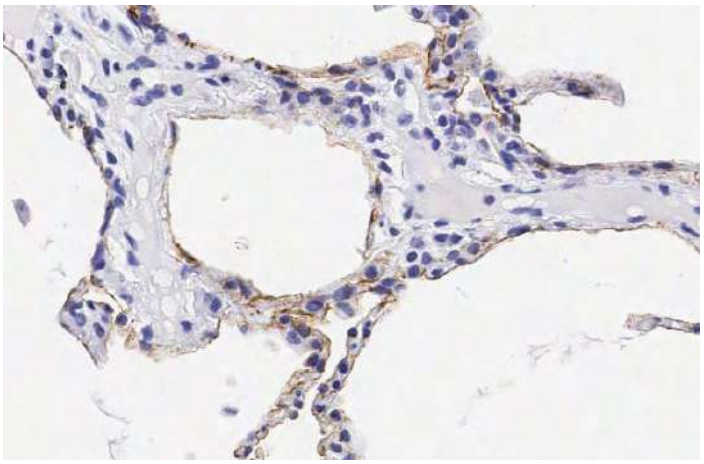
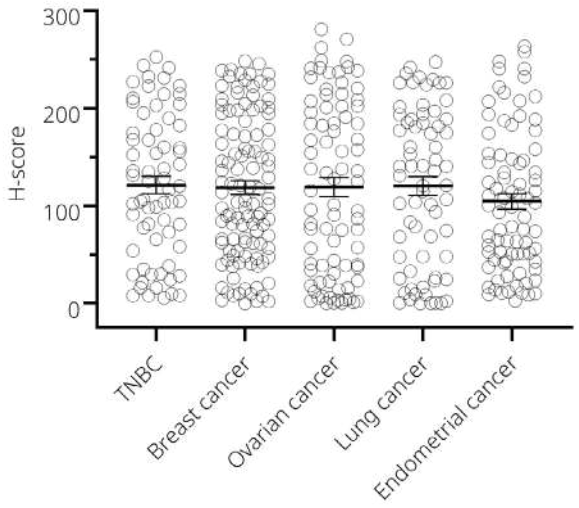


Figure 2. TROP2 expression in human normal tissue. IHC staining of normal human testis, skin, ovary, and lung tissue using anti-TROP2 (ab214488) or rabbit IgG–isotype control antibody (ab172730). Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope. Expression was detected in the prostate, pancreas, kidney and tonsil, and absent from stomach, colon, small intestine, spleen, cerebrum, testis, skeletal muscle and heart muscle (not shown).

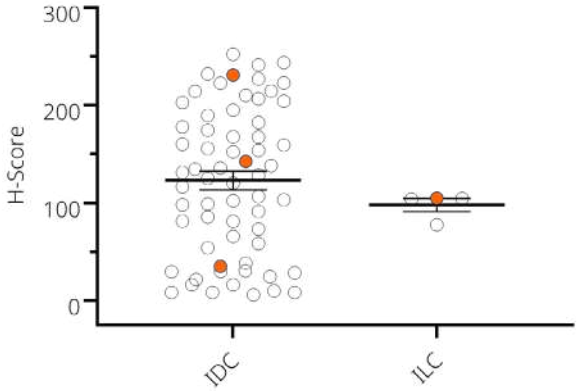
TROP2 expression in cancer (BOND™ RX)

The TROP2 expression profile was similar in the analyzed cancer tissue microarrays (TMAs), with endometrial cancer having a slightly lower mean H-score (a). The H-score of cohorts of cancer subtypes was also evaluated separately in scatter plots (with SD) (b-f).

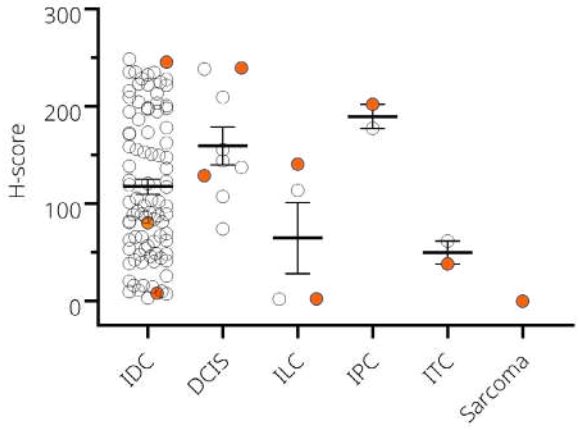
a) TROP2 expression in selected cancer TMAs



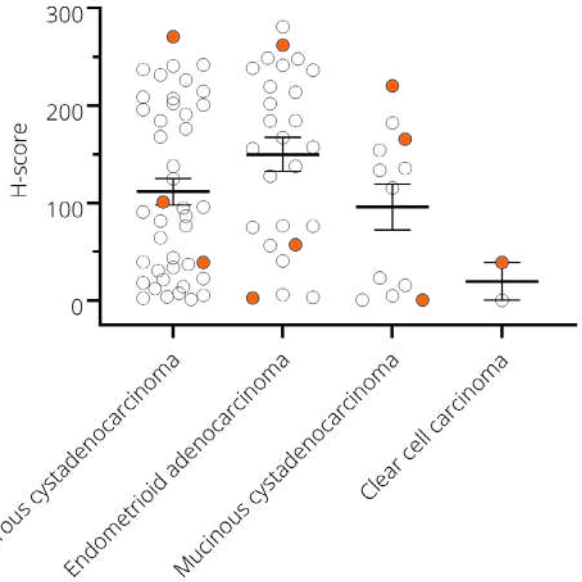
b) TROP2 expression in TNBC



c) TROP2 expression in breast cancer



d) TROP2 expression in ovarian cancer



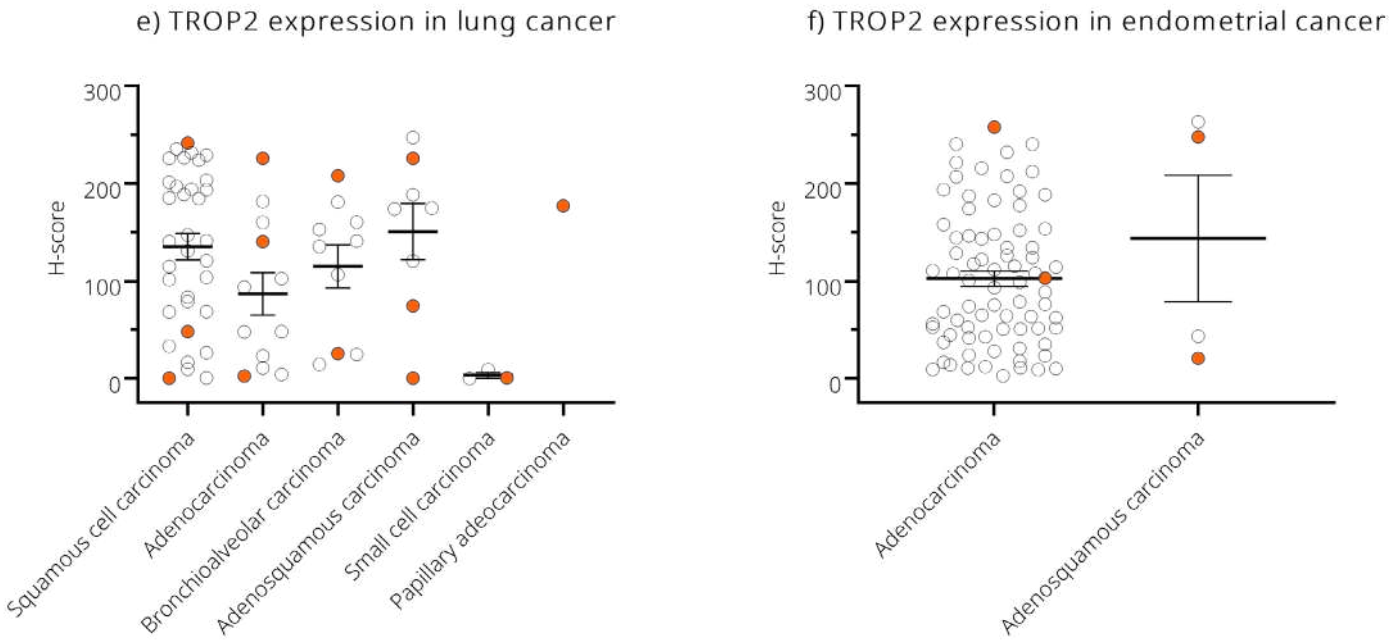
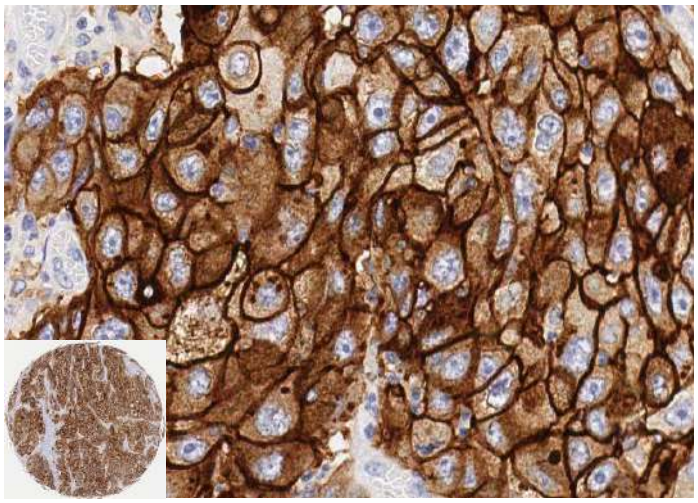


Figure 3. TROP2 protein expression in a selection of cancer TMAs.
(a) Mean (with SD) H-score (maximum value 300) of TROP2 expression in TMA cores.
(b) TROP H-score from 67 triple negative breast cancer (TNBC), invasive ductal carcinoma (IDC) (63), invasive lobular carcinoma (ILC) (4).
(c) TROP2 H-score from 111 TMA cores of breast cancer, (invasive ductal carcinoma (IDC) (91), ductal carcinoma in situ (DCIS) (9), invasive lobular carcinoma (ILC) (4), fibroadenoma (2), invasive papillary carcinoma IPC (2), invasive tubular carcinoma ITC (2), sarcoma (1).
(d) TROP2 H-score from 82 TMA cores of ovarian cancer (serous cystadenocarcinoma (42), endometrioid adenocarcinoma (26), mucinous cystadenocarcinoma (12), clear cell carcinoma (2), cystadenoma (1).
(e) TROP2 H-score from 68 cores of lung cancer (squamous cell carcinoma (34), adenocarcinoma (12), bronchioalveolar carcinoma (10), adenosquamous carcinoma (8), small cell carcinoma (3), papillary adeocarcinoma (3).
(f) TROP2 H-score from 81 cores of endometrial cancer (adenocarcinoma (77), adenosquamous carcinoma (4).
The IHC images corresponding to orange data points are shown in Figures 4-8.

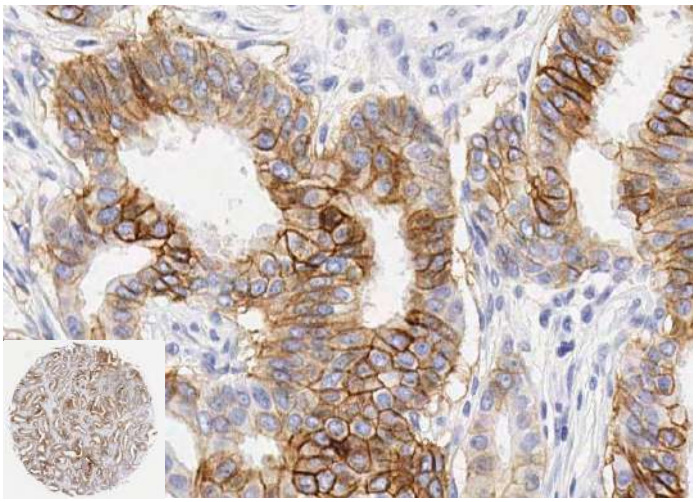
TROP2 expression in triple negative breast cancer TMA (BOND™ RX)

Below are the representative images of individual triple negative breast cancer cases showing strong to weak TROP2 expression.

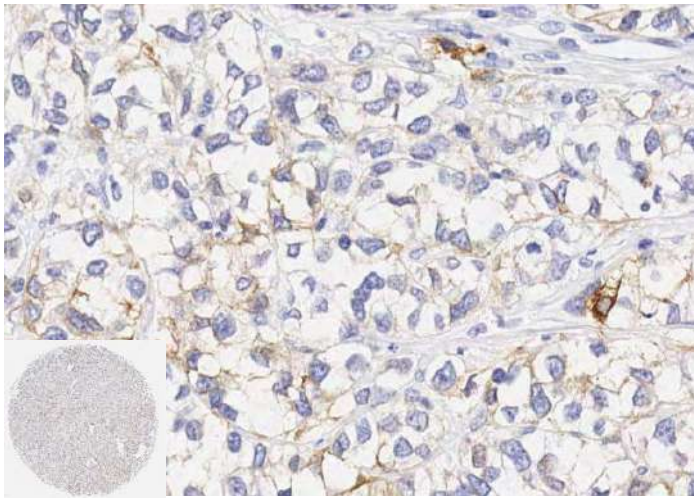
IDC
(H-score 231.19)



IDC
(H-score 142.59)



IDC
(H-score 34.95)



ILC
(H-score 104.87)

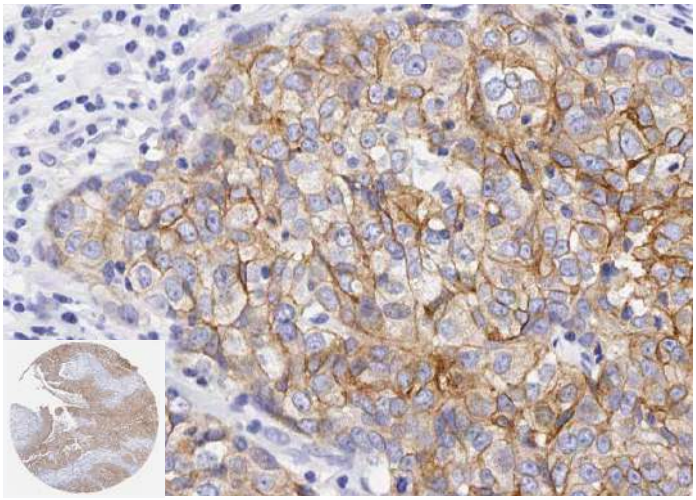
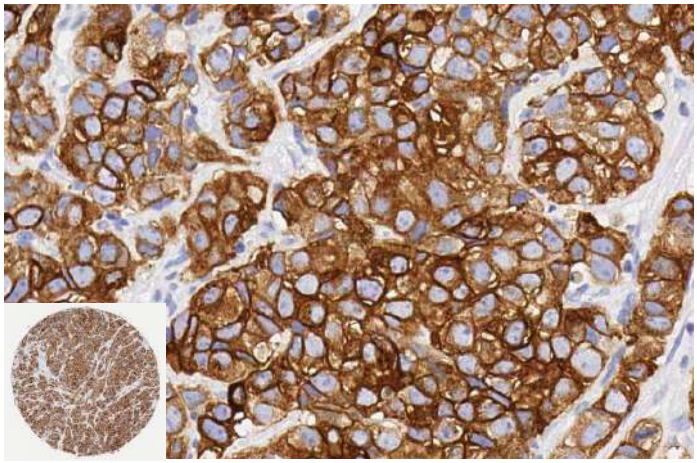


Figure 4. TROP2 expression in triple negative breast cancer. IHC images show strong, moderate or weak TROP2 staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

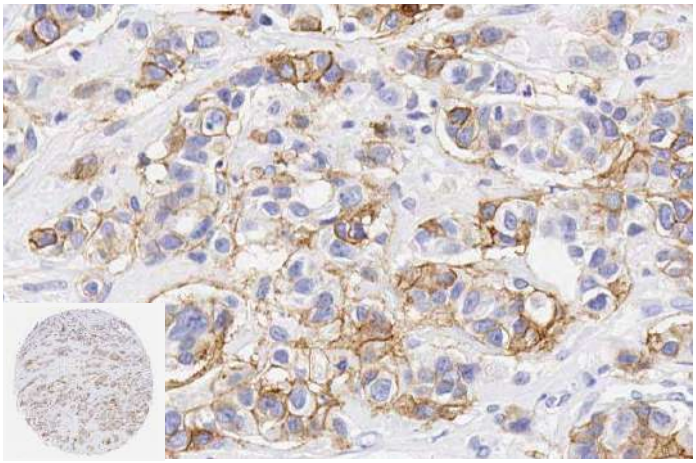
TROP2 expression in breast cancer TMA (BOND™ RX)

Below are the representative images of individual breast cancer cases showing strong to weak TROP2 expression.

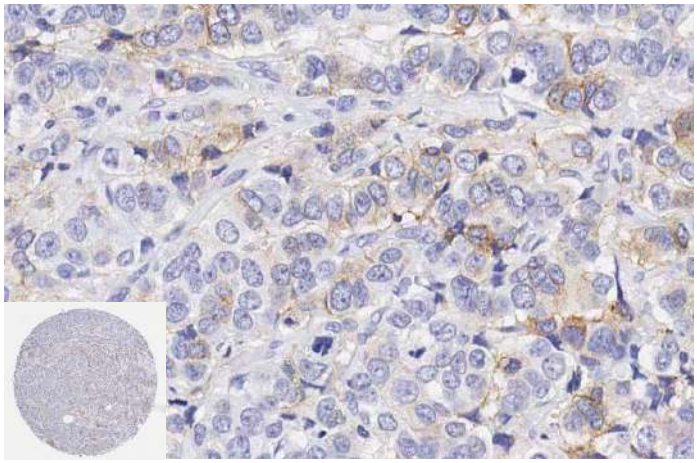
IDC
(H-score 245.47)



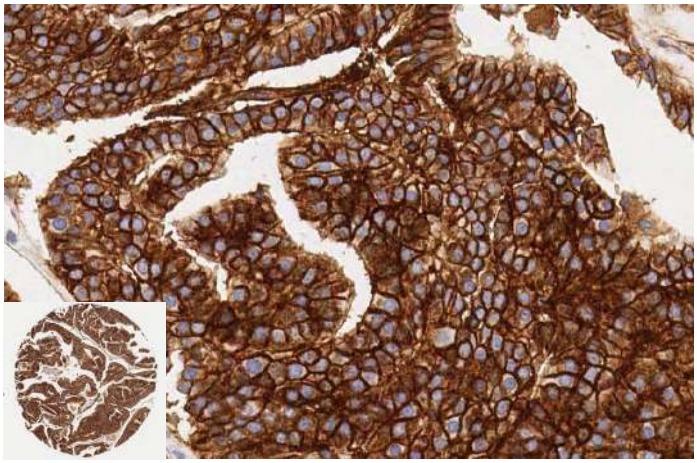
IDC
(H-score 80.62)



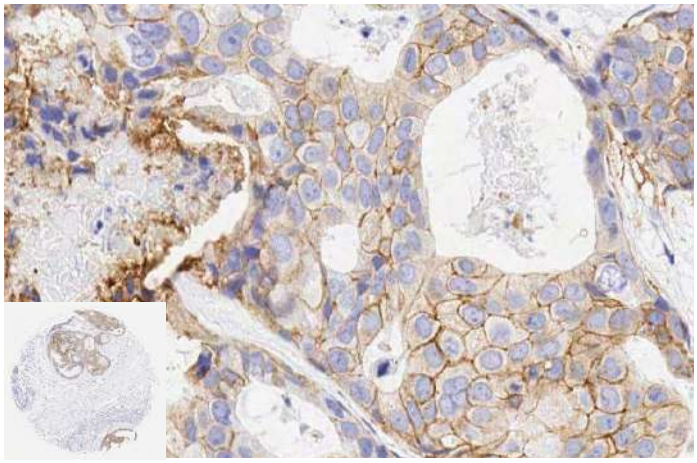
IDC
(H-score 8.23)



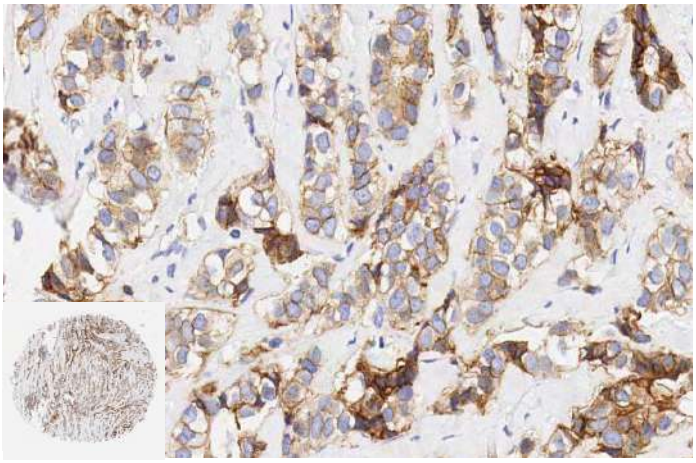
DCIS
(H-score 239.38)



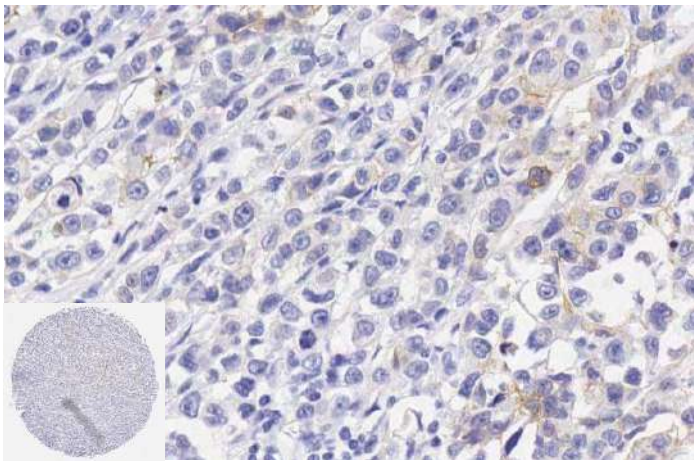
DCIS
(H-score 129.08)



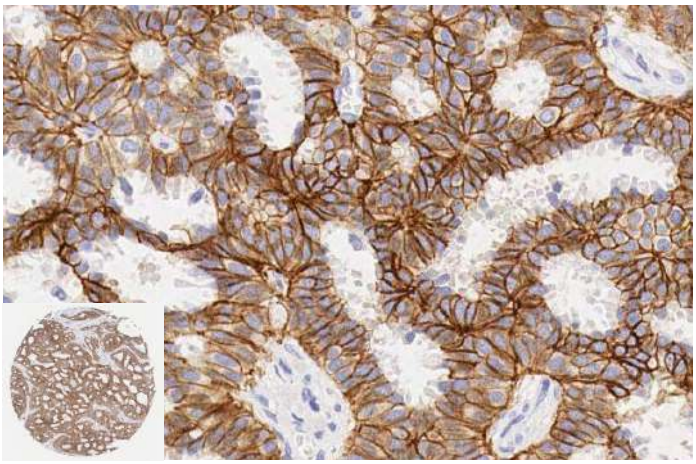
ILC
(H-score 140.60)



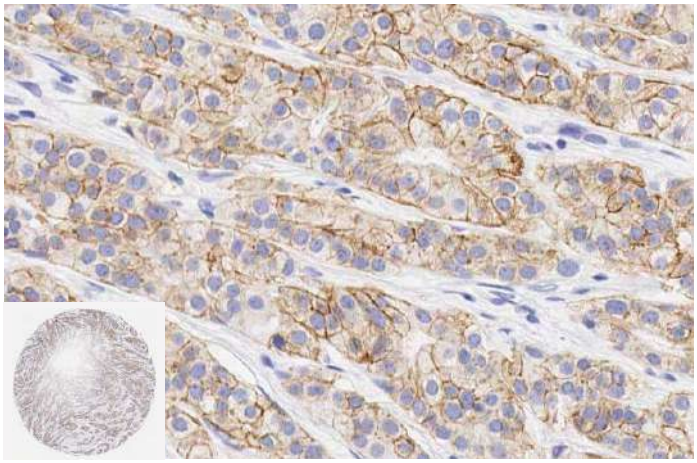
ILC
(H-score 2.4)



IPC
(H-score 202.17)



ITC
(H-score 38.3)



Sarcoma
(H-score 0.1)

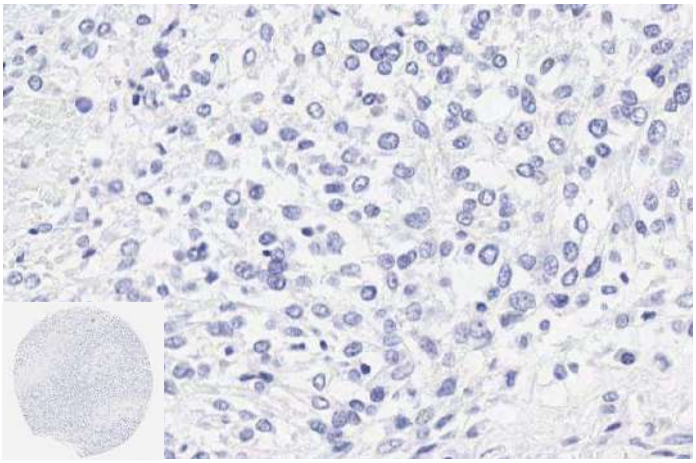
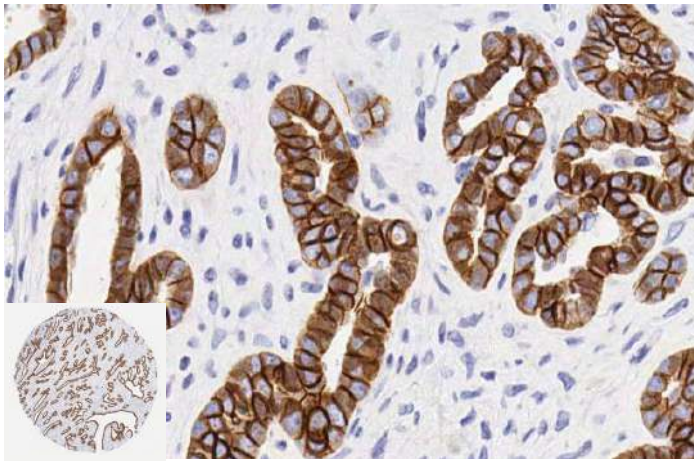


Figure 5. TROP2 expression in breast cancer. IHC images show strong, moderate or weak TROP2 staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

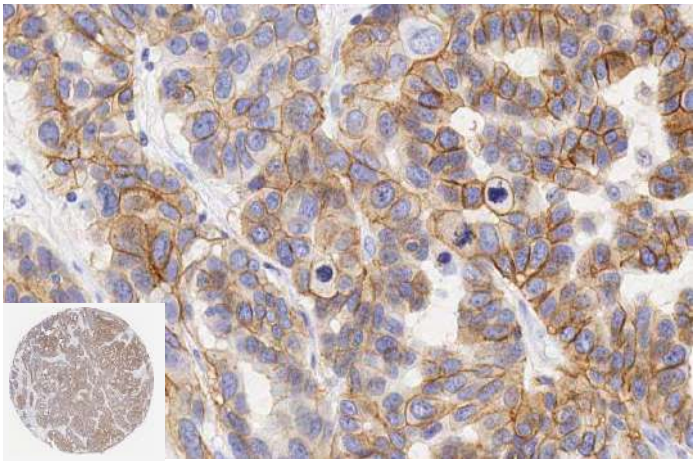
TROP2 expression in ovarian cancer TMA (BOND™ RX)

Below are the representative images of strong to weak TROP2 expression in individual cases of ovarian cancer.

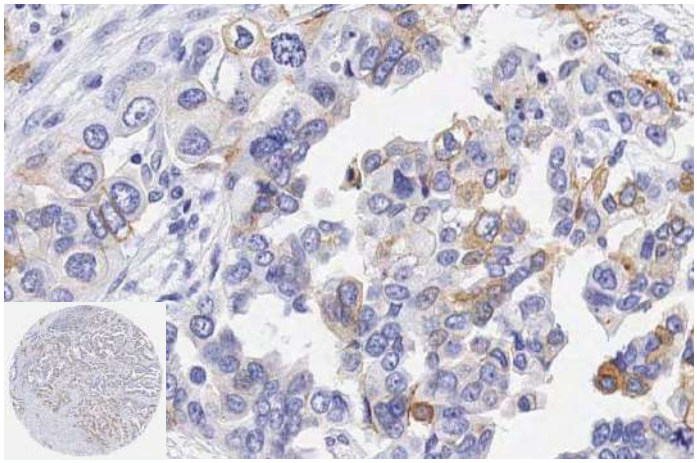
Serous cystadenocarcinoma
(H-score 270.54)



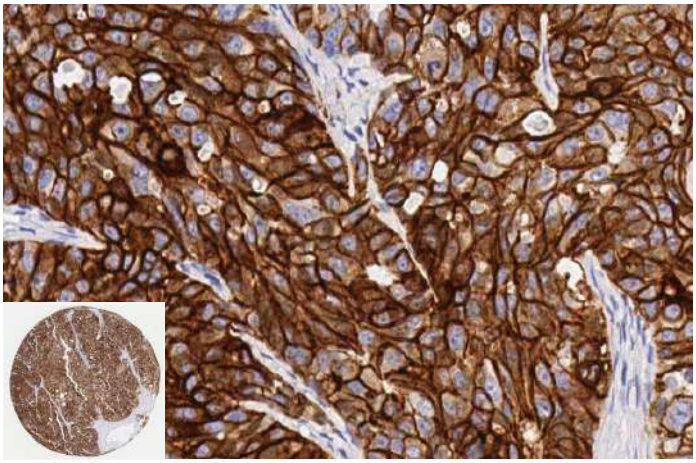
Serous cystadenocarcinoma
(H-score 100.96)



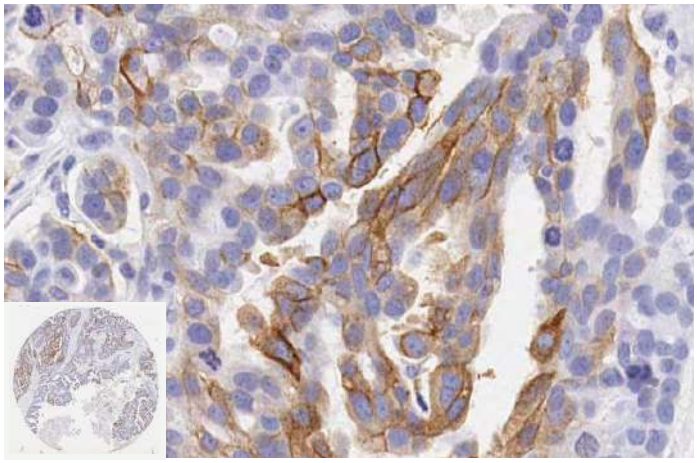
Serous cystadenocarcinoma
(H-score 39.13)



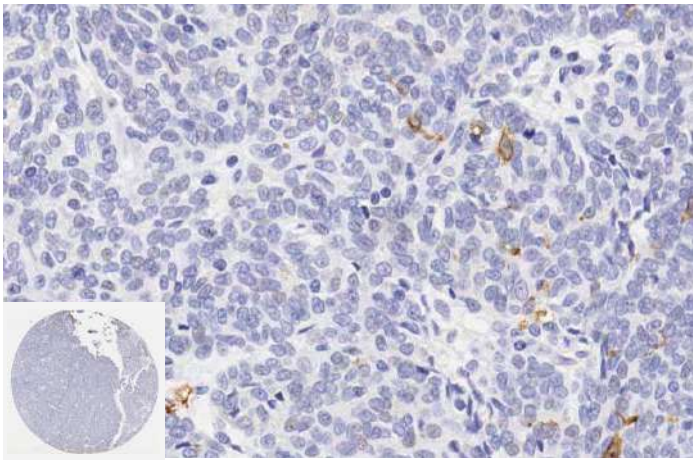
Endometrioid adenocarcinoma
(H-score 261.90)



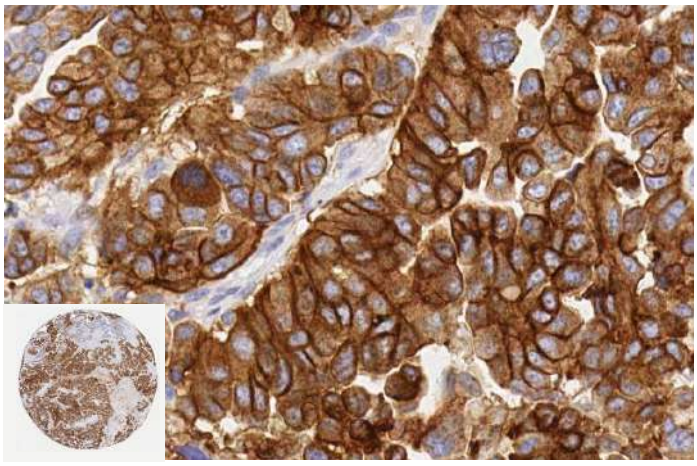
Endometrioid adenocarcinoma
(H-score 57.15)



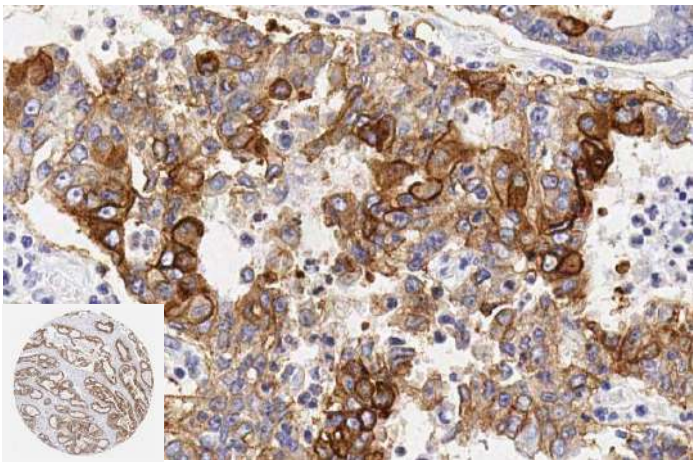
Endometrioid adenocarcinoma
(H-score 2.75)



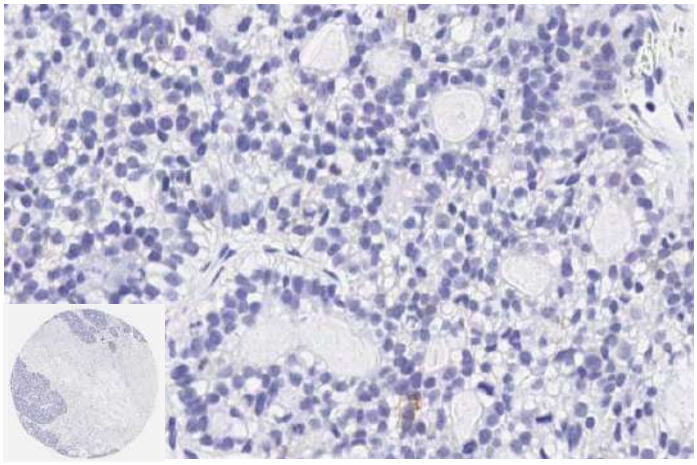
Mucinous cystadenocarcinoma
(H-score 220.33)



Mucinous cystadenocarcinoma
(H-score 165.60)



Mucinous cystadenocarcinoma
(H-score 0.66)



Clear cell carcinoma
(H-score 39.06)

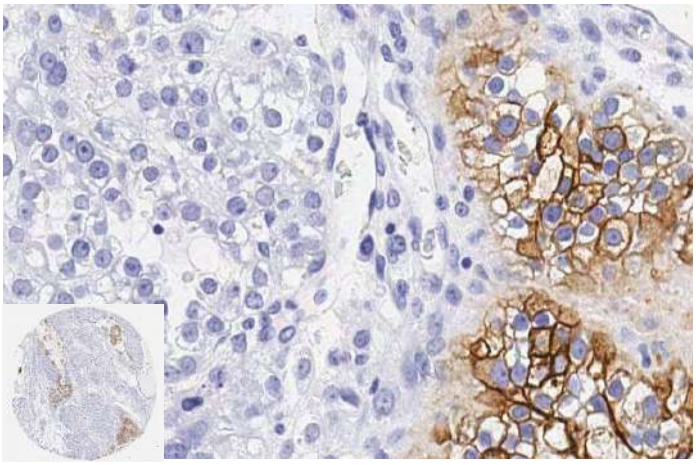
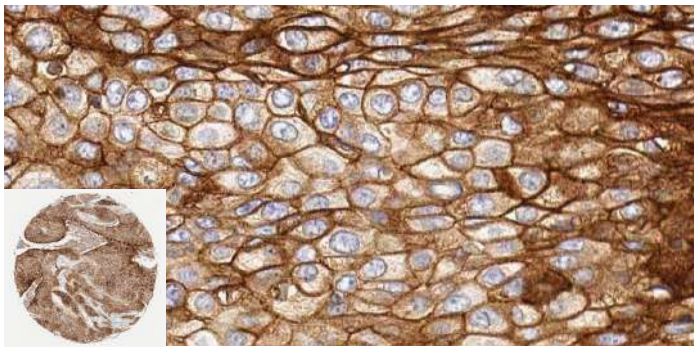


Figure 6. TROP2 expression in ovarian cancer. IHC images show strong, moderate or weak TROP2 staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

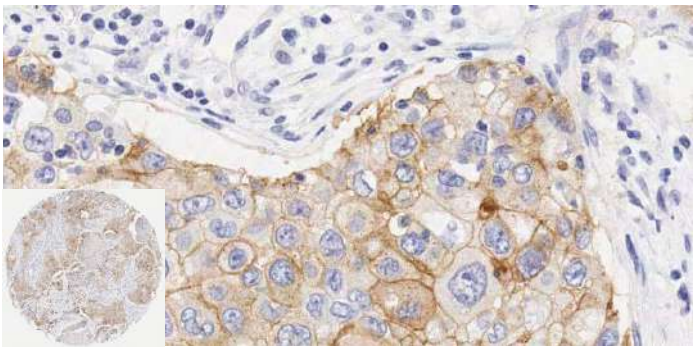
TROP2 expression in lung cancer TMA (BOND™ RX)

Below are the representative images of individual cases of lung cancer showing strong to weak TROP2 expression.

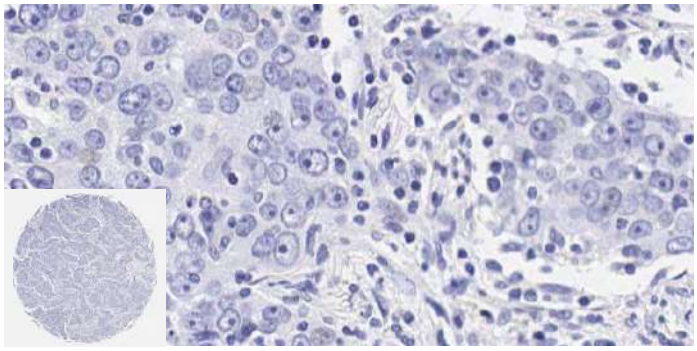
Squamous cell carcinoma
(H-score 241.58)



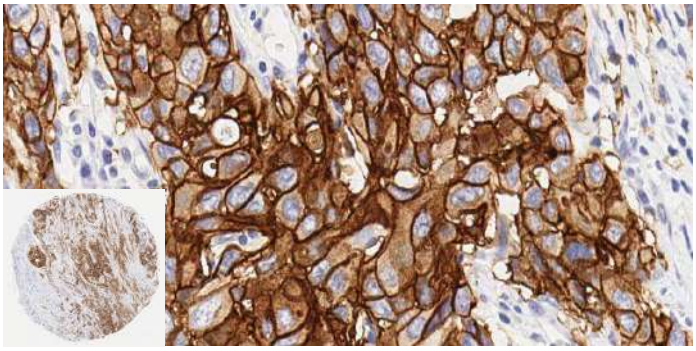
Squamous cell carcinoma
(H-score 48.03)



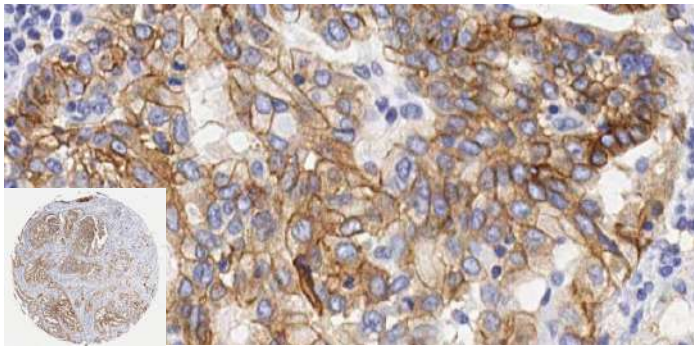
Squamous cell carcinoma
(H-score 0.31)



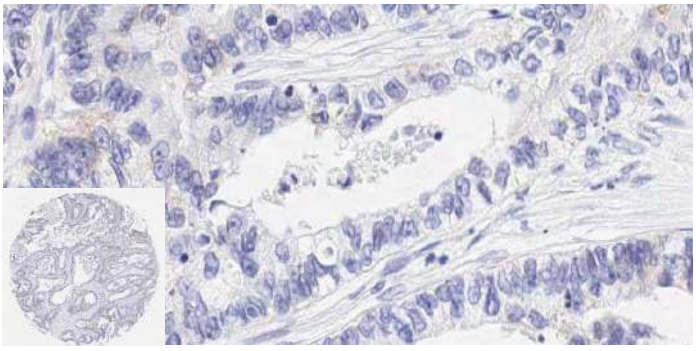
Adenocarcinoma
(H-score 225.81)



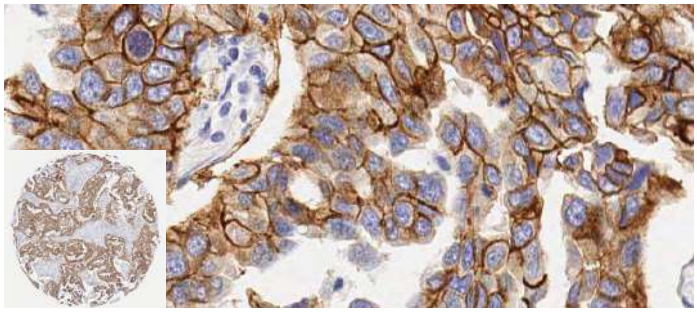
Adenocarcinoma
(H-score 140.40)



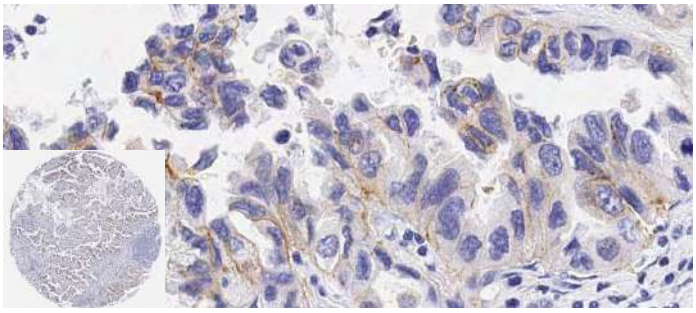
Adenocarcinoma
(H-score 2.50)



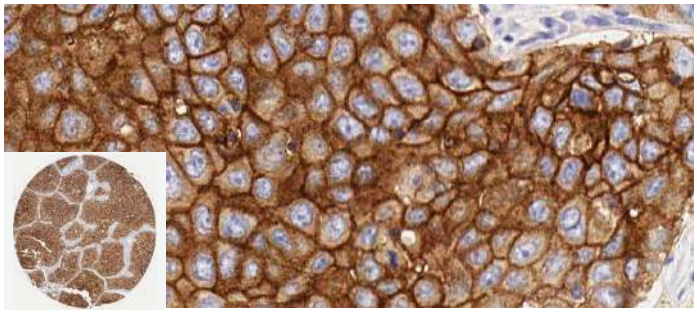
Bronchioalveolar carcinoma
(H-score 207.93)



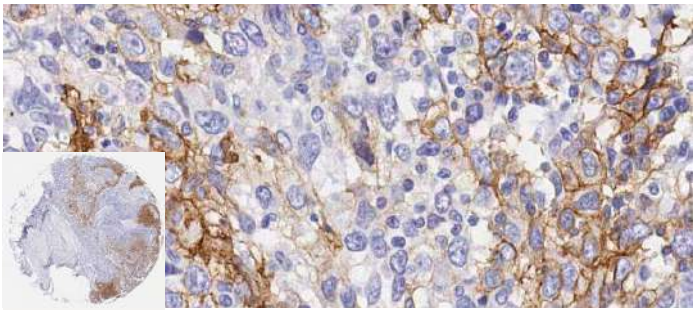
Bronchioalveolar carcinoma
(H-score 25.55)



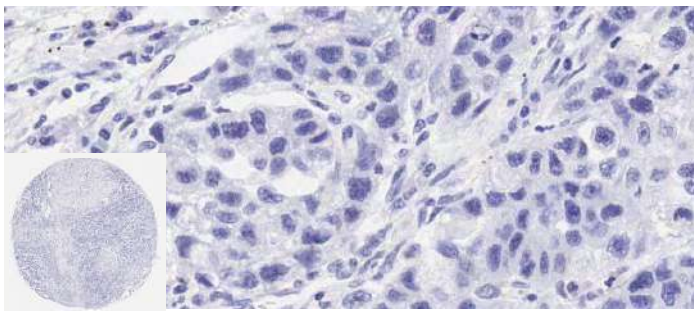
Adenosquamous carcinoma
(H-score 225.82)



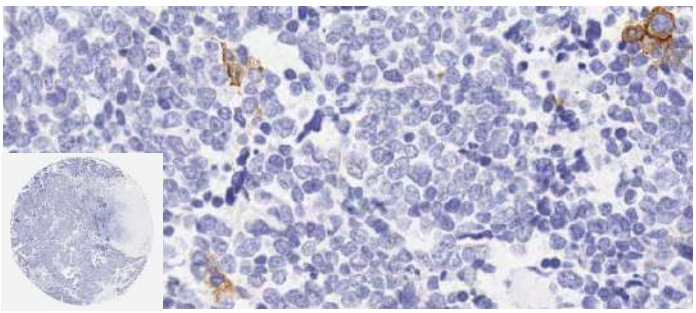
Adenosquamous carcinoma
(H-score 74.56)



Adenosquamous carcinoma
(H-score 0.4)



Small cell carcinoma
(H-score 0.66)



Papillary adeocarcinoma
(H-score 177.20)

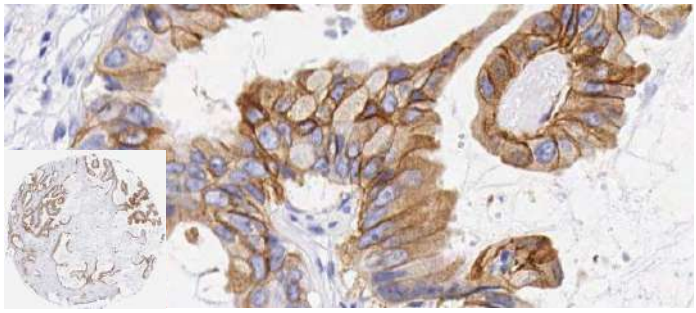
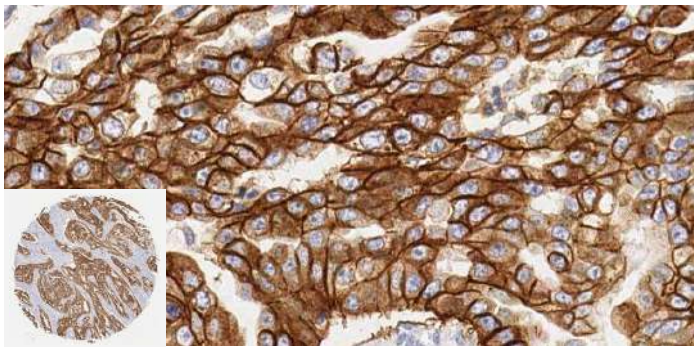


Figure 7. TROP2 expression in lung cancer. IHC images show strong, moderate or weak TROP2 staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

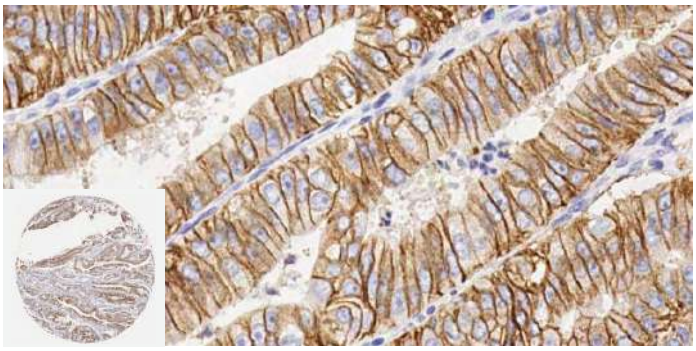
TROP2 expression in endometrial cancer TMA (BOND™ RX)

Below are the representative images of individual endometrial cancer cases showing strong to weak TROP2 expression.

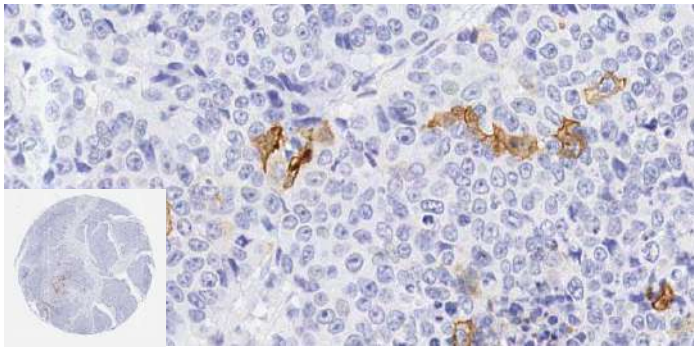
Adenocarcinoma
(H-score 257.9)



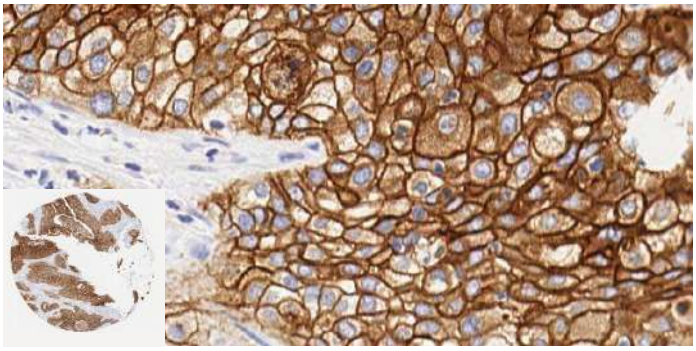
Adenocarcinoma
(H-score 102.96)



Adenocarcinoma
(H-score 2.77)



Adenosquamous carcinoma
(H-score 247.97)



Adenosquamous carcinoma
(H-score 20.59)

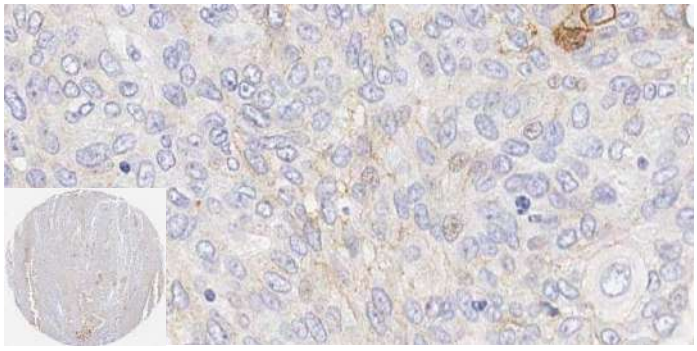
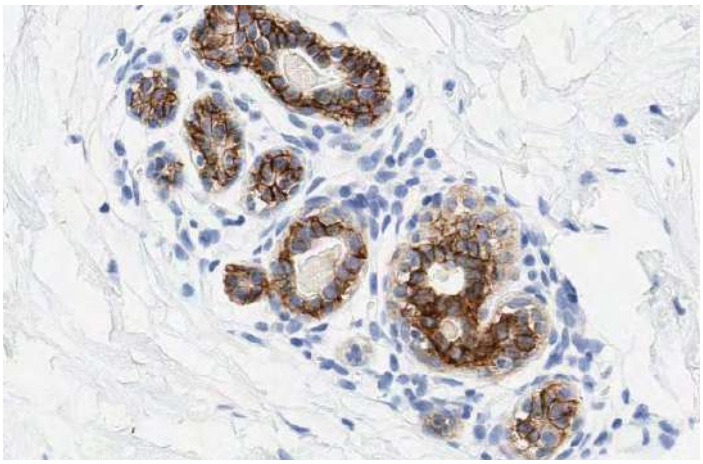
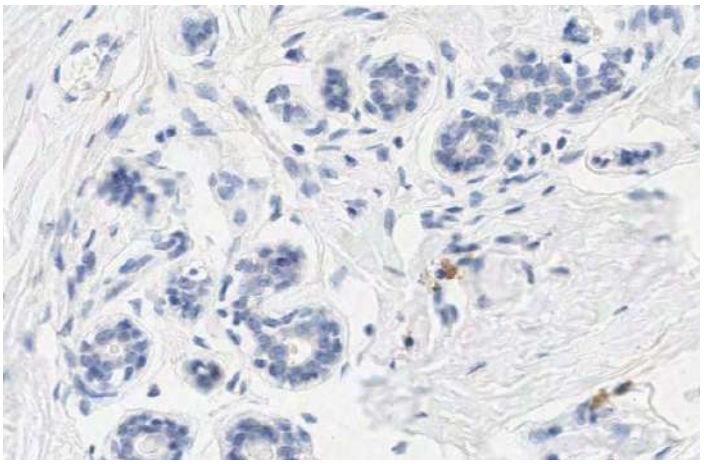
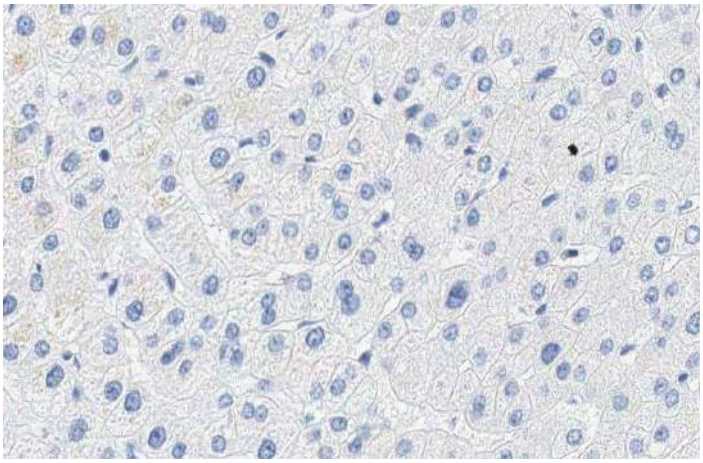
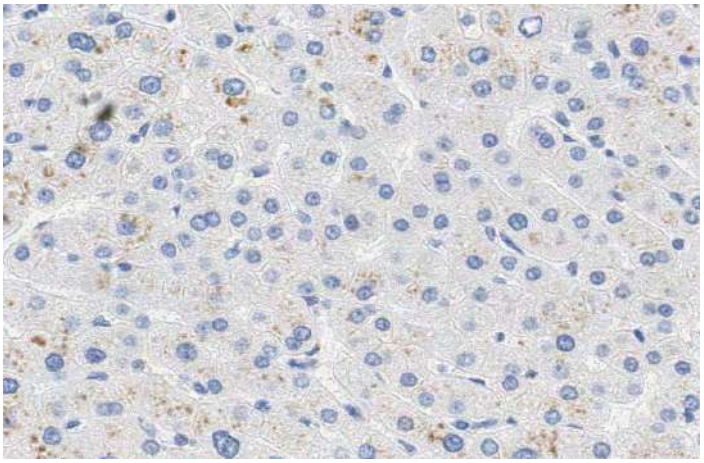


Figure 8. TROP2 expression in endometrial cancer. IHC images show strong, moderate or weak TROP2 staining intensity in brown. Nuclear hematoxylin counterstain in blue. Slides were scanned at 20x on the Aperio® AT2 and imaged at 20x (whole core insets at 5x) on Aperio® ImageScope.

TROP2 expression in human normal and cancer tissues (DISCOVERY ULTRA)

Below are the representative images of selected human normal and cancer tissues stained on the Discovery ULTRA platform. In normal tissue, TROP2 expression was detected in skin, breast, and lung tissues and was absent from the liver. TROP2 expression was also present in breast adenocarcinoma fibroadenoma.

TROP2	Isotype Control
Breast	
	
Liver	
	

TROP2

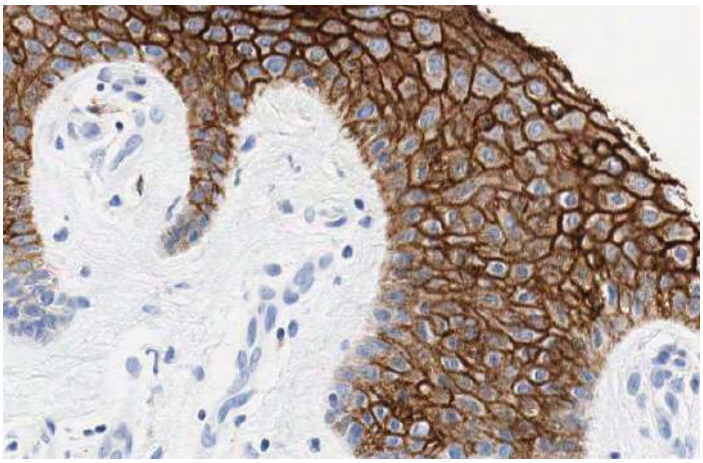
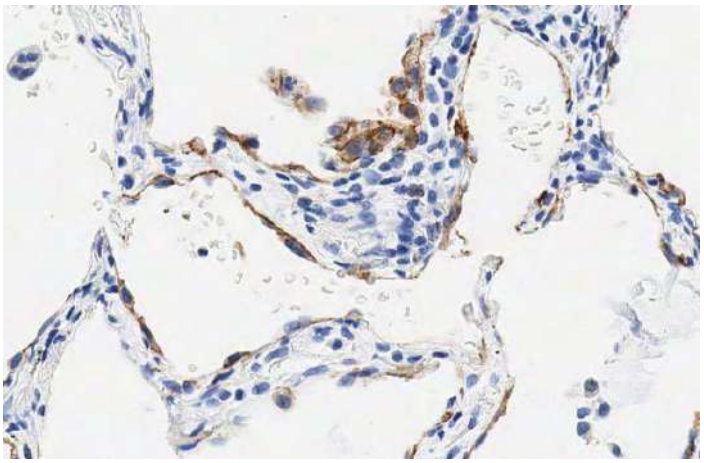
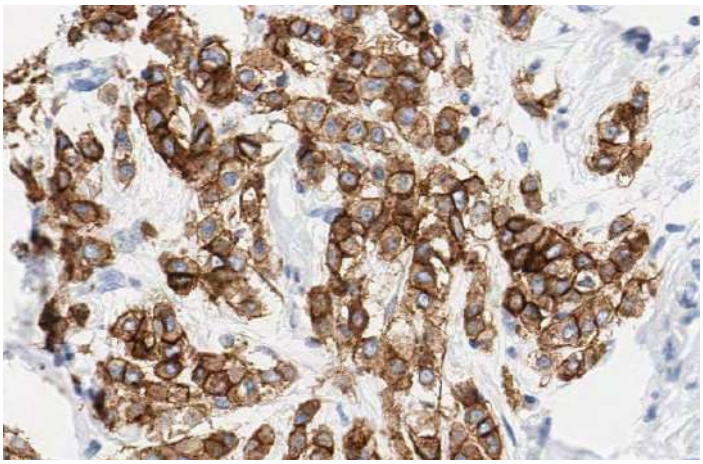
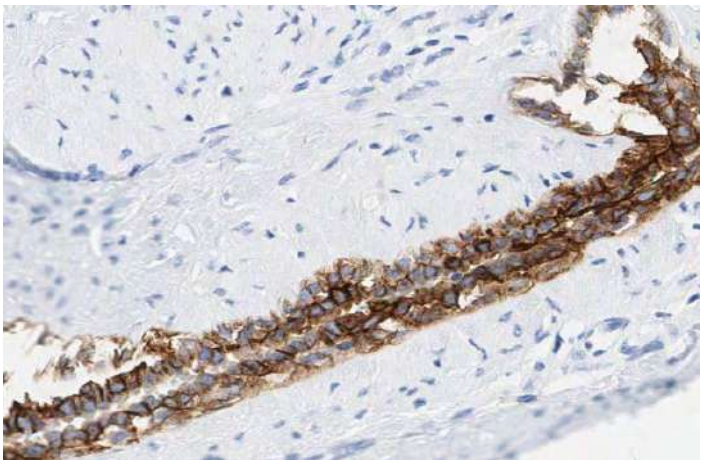
Skin	Lung
	
Breast adenocarcinoma	Breast fibroadenoma
	

Figure 9. TROP2 expression in human normal tissue. IHC staining of normal human testis, skin, ovary and lung tissue using anti-TROP2 (ab214488) or rabbit IgG–isotype control antibody, (1ug/ml, ab172730) using the DISCOVERY ULTRA platform. Positive staining in brown; hematoxylin nuclear counterstain in blue. Slides were scanned at 20x on Aperio® AT2 and imaged at 20x on Aperio® ImageScope.

References

1. Lin, Jau-Chen et al. "TROP2 is epigenetically inactivated and modulates IGF-1R signalling in lung adenocarcinoma." *EMBO molecular medicine* vol. 4,6 (2012): 472-85. doi:10.1002/emmm.201200222

2. Fornaro, M et al. "Cloning of the gene encoding Trop-2, a cell-surface glycoprotein expressed by human carcinomas." *International journal of cancer* vol. 62,5 (1995): 610-8. doi:10.1002/ijc.2910620520

3. Ripani, E et al. "Human Trop-2 is a tumor-associated calcium signal transducer." International journal of cancer vol. 76,5 (1998): 671-6. doi:10.1002/(sici)1097-0215(19980529)76:5<671::aid-ijc10>3.0.co;2-7

4. Trerotola, M et al. "Upregulation of Trop-2 quantitatively stimulates human cancer growth." *Oncogene* vol. 32,2 (2013): 222-33. doi:10.1038/onc.2012.36

5. Cardillo, Thomas M et al. "Humanized anti-Trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: preclinical studies in human cancer xenograft models and monkeys." *Clinical cancer research: an official journal of the American Association for Cancer Research* vol. 17,10 (2011): 3157-69. doi:10.1158/1078-0432.CCR-10-2939

6. Cubas, Rafael et al. "Trop2: a possible therapeutic target for late stage epithelial carcinomas." *Biochimica et biophysica acta* vol. 1796,2 (2009): 309-14. doi:10.1016/j.bbcan.2009.08.001

7. Lipinski, M et al. "Human trophoblast cell-surface antigens defined by monoclonal antibodies." *Proceedings of the National Academy of Sciences of the United States of America* vol. 78,8 (1981): 5147-50. doi:10.1073/pnas.78.8.5147

8. Dum, David et al. "Trophoblast Cell Surface Antigen 2 Expression in Human Tumors: A Tissue Microarray Study on 18,563 Tumors." *Pathobiology : journal of immunopathology, molecular and cellular biology* vol. 89,4 (2022): 245-258. doi:10.1159/000522206

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danaher