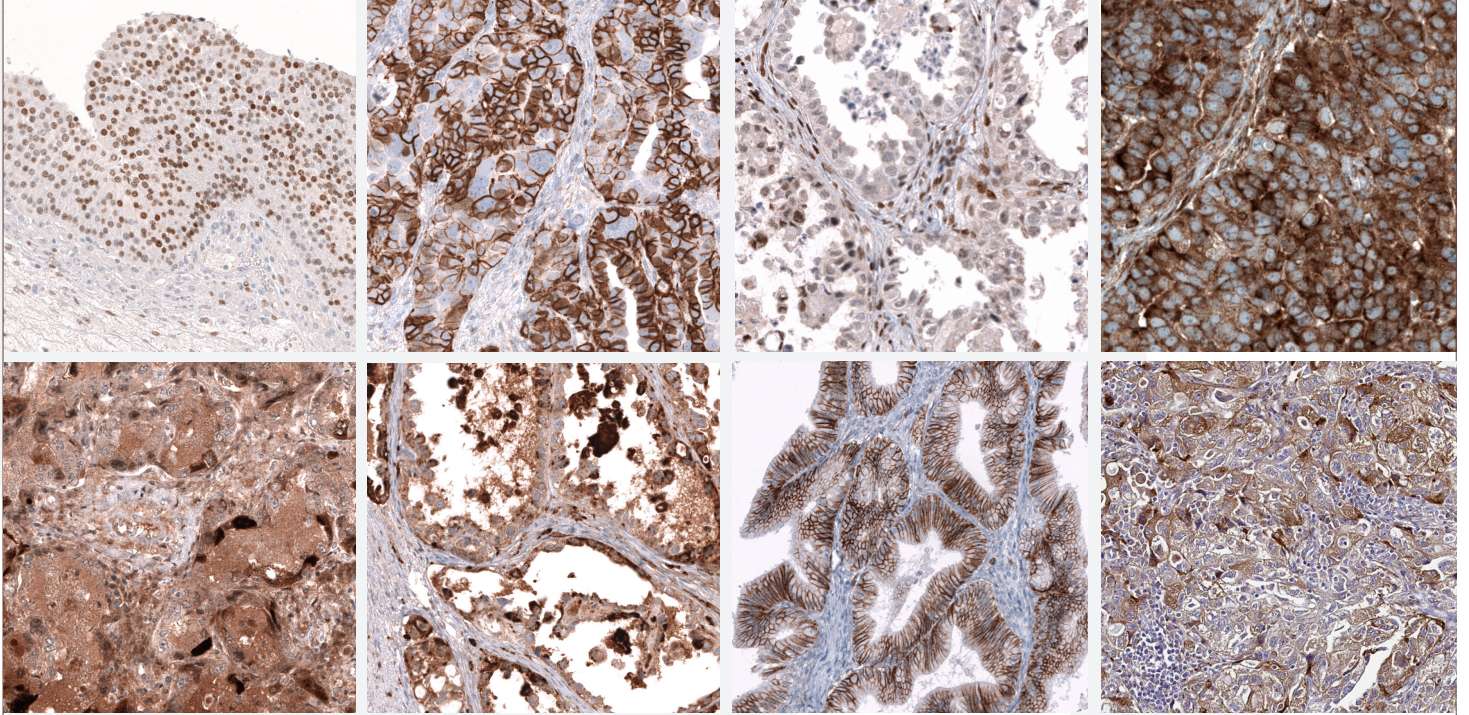
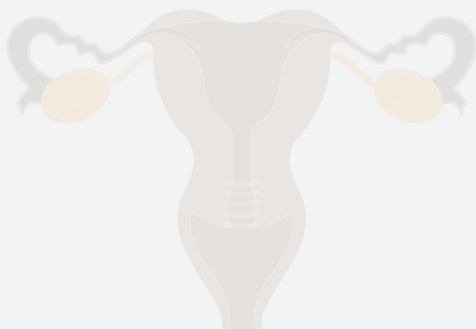


# PRIMARY ANTIBODIES FOR CANCER RESEARCH



# OVARIAN CARCINOMAS



# Ovarian Carcinomas

Gynecologic cancers originate in the female reproductive organs. The 5 main types of gynecologic cancers are cervical, ovarian, uterine, vaginal, and vulvar.

Of these, ovarian cancer is the deadliest form causing more deaths than any other cancer of the female reproductive system with around two-thirds of patients diagnosed with advanced disease due to late presentation. Furthermore, around 90% of patients develop recurrence and eventually become chemoresistant.

Therefore, there is a high demand to identify biomarkers specific to this disease for screening for early detection, as well as new therapeutic targets. The capacity of these biomarkers to predict the existence, stages, and associated therapeutic efficacy of ovarian cancer would enable improvements in the early diagnosis and survival of ovarian cancer patients.

Atlas Antibodies continues searching for better early detection markers and new therapeutic targets. This white paper presents our selected PrecisA Monoclonals™ and TripleA Polyclonals™ targeting ovarian cancers.

Ovarian cancer is the eighth most common cancer among women and the fifth cause of cancer-related death in women, with a 5-year survival of only 30–50%.

It remains the deadliest gynecologic malignancy in the western world and is most often diagnosed at a rarely curable late stage. The mean age of diagnosis is 64 years old. Five to ten % of ovarian cancers are familial.

Ovarian cancer is often asymptomatic in the early stages. As a result, most patients with ovarian cancer are diagnosed at an advanced clinical stage when curative therapy is no longer possible.

By the time of discovery, approximately 70% of the tumors have spread beyond the ovary and are rarely curable by surgical resection or surgery combined with postoperative chemotherapy and/or radiation therapy.

Early detection of ovarian cancer would improve the 5-year survival rate, from only 20% when the cancer is discovered in stage IV to close to 90% in stage I (Torre 2018).

Thus far, the primary tumor biopsy followed by an immunohistochemical analysis

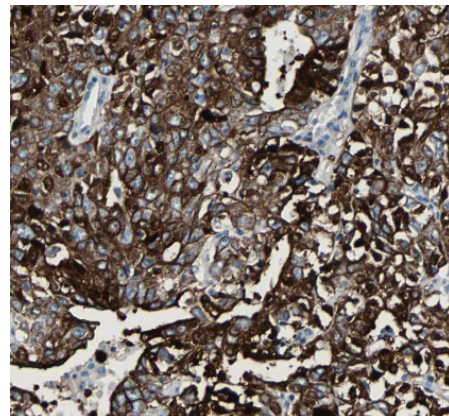
remains the gold standard to identify molecular alterations associated with the tumors and potentially prognostic and predictive biomarkers.

However, in ovarian cancer diagnostic, none of the biomarkers available today are accurate enough to identify early-stage ovarian cancer (sensitivity) without including a considerable fraction of false positives (specificity).

Therefore, there is a critical need to revisit the existing and identify new biomarkers enabling the development of novel and more effective predictors for ovarian cancer diagnosis and prognosis.

## Anti-KRT7 (HPA007272)

Endometrial Carcinoma



KRT7 is a gene associated with unfavorable prognosis in ovarian cancer. The image shows the immunohistochemical staining of endometrial carcinoma using the Anti-KRT7 (HPA007272) polyclonal antibody.

**Cover: representative immunostainings on human ovarian carcinoma using Atlas Antibodies' primary antibodies.**

**Up:** anti-NCAM1 (AMAb91807, ovarian papillary serous cystadenocarcinoma); anti-FOXO2 (AMAb91808, ovary follicle granulosa cells carcinoma); anti-TFE3 (AMAb91798, clear cell ovarian carcinoma); anti-GSPT1 (AMAb91849, ovarian papillary carcinoma).

**Bottom:** anti-WFDC2 (AMAb91821, endometrioid carcinoma); anti-NAPSA (AMAb91825, clear cell carcinoma); anti-CD44 (AMAb91847, low-grade serous carcinoma); anti-MRC2 (HPA041991, ovarian carcinoma).

# Ovarian cancer is a heterogeneous disease

Heterogeneity represents a hallmark of many cancers, including ovarian cancer that comprises a histologically and genetically broad range of tumors (figure 1).

Ovarian cancer (often used as a generic term to define any cancer involving the ovaries) includes cancers of the ovary, fallopian tubes, and peritoneum due to the origination from similar tissue types and similar clinical management and treatment but with distinct clinicopathological, molecular features and prognosis.

Despite there being a variety of ovarian cancer subtypes, these are often treated as a single disease.

Efforts have been made to characterize these subtypes and identify tumoral pathways and potential biomarkers for therapeutic strategies.

# Types of ovarian cancer

Different types of ovarian cancer tumors are named after the type of cell they originate from, i.e., the three main cell types that make up the ovary: germ cells, stromal cells and epithelial cells.

- **Small Cell Carcinoma**

This is a sporadic ovarian cancer, and it is not sure whether the cells in small cell carcinoma are from ovarian epithelial cells, sex-cord stromal cells, or germ cells.

- **Germ Cell Carcinoma**

Comes from the reproductive cells of the ovaries. Germ cell tumors begin in the reproductive cells (egg or sperm) of the body. Germ cell ovarian cancer is rare, however, it usually occur in teenage girls or young women and most often affect just one ovary.

- **Sex Cord Stromal Carcinoma**

Stromal cells are among the three most common cell types to be affected by ovarian cancer. Ovarian stromal tumors are sporadic and develop in the ovaries' structural connective tissue cells that

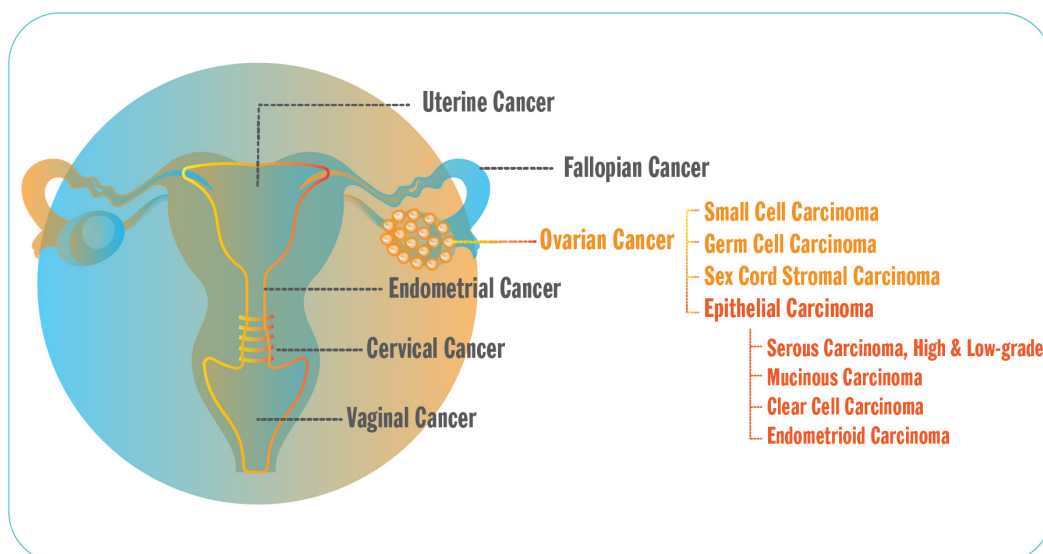
produce the female hormones estrogen and progesterone. Ovarian granulosa cell tumors represent approximately 2% to 5% of all ovarian cancers. They are the most common type of ovarian sex-cord stromal tumors

- **Epithelial Carcinoma**

Epithelial ovarian cancer is the most common ovarian cancer that comes from the surface of the ovary (the epithelium) and includes:

- **Endometrial Carcinoma (EC)**
- **Clear Cell Carcinoma (CCC)**
- **Mucinous Carcinoma (MC)**
- **Serous Carcinoma (SC)** which include high-grade serous carcinoma (HGSC) and low-grade serous carcinoma (LGSC)

Figure 2 shows a schematic of epithelial ovarian cancer subtypes including prognosis, common mutations and immunophenotypes for tissue biopsy.



**Figure 1. Schematic of female genital tract and ovarian cancer types.**

Ovarian cancers are named after the type of cell they come from, i.e. the three main cell types that make up the ovary: germ cell, stromal and epithelial.

Epithelial carcinoma include small cell carcinoma, germ cell carcinoma, sex cord stromal carcinoma and serous carcinoma (high-grade and low-grade).

# Epithelial ovarian carcinoma subtypes

Epithelial ovarian carcinoma is the most common type of ovarian cancer. This cancer develops in the epithelial tissue, the thin lining that covers the outside of an ovary. Cancer may also form in the lining of a fallopian tube. Or it can begin in the peritoneum, the tissue that covers your abdominal organs.

Medical experts consider fallopian tube cancers and primary peritoneal cancers to be epithelial ovarian cancers. The diseases share many similarities, including treatments.

Epithelial carcinoma include small cell carcinoma, germ cell carcinoma, sex cord stromal carcinoma and serous carcinoma (high-grade and low-grade).

About 3 out of 4 epithelial ovarian cancers are high-grade serous

ovarian carcinomas (HGSC). Cancer cells that are high-grade grow and spread faster than those that are low-grade.

HGSC grows slowly at first. It starts in the fallopian tubes and it may take up to six and a half years to reach the ovaries. However, once the cancer is in the ovaries, it spreads quickly. The cancer often affects the peritoneum and other parts of the body.

Nearly 70% of HGSCs are stage 3 or 4 at the time of diagnosis. This means the cancer has spread outside of the original tumor and is now metastatic cancer.

Over the past several years, researchers have developed a streamlined classification scheme for the five major categories of epithelial ovarian cancers based on histology, origin, degree of differentiation, and molecular features.

Primary epithelial ovarian carcinoma is sub-classified into serous, mucinous, endometrioid and clear cell subtypes (figure 2).

Epithelial ovarian carcinoma subtypes have distinct expression profiles. However, the biomarker expression profile within a given subtype is consistent across stages.

## Stages of ovarian cancers

**Stage 1:** Ovarian cancer in stage 1 is contained in one or both ovaries. It hasn't spread to nearby lymph nodes.

**Stage 2:** Ovarian cancer in stage 2 is in one or both ovaries and has spread to other organs in the pelvis. These organs might include the uterus, bladder, rectum, or fallopian tubes.

**Stage 3:** Ovarian cancer has spread beyond the ovaries and pelvis and into the abdomen, abdominal lining, or nearby lymph nodes.

**Stage 4:** Stage 4 ovarian cancer is the terminal stage. Cancer in this stage has spread beyond the abdomen. It may have reached the spleen, lungs, or liver.

Epithelial Ovarian Cancer Subtypes	Cell Line of Origin	% of all Ovarian Carcinomas	Prognosis & Presentation	Common Mutations & Molecular Aberrations	Immunophenotype (IHC markers)
High-Grade Serous Carcinoma (HGSC)	Fallopian Tube Epithelium	~ 70%	Poor: presents at older age and high stage.	ubiquitous TP53 mut high BRCA1/2 mut genomic instability	CK7+ PAX8+ WT1+ ER+ CK20-, FOXL2- CALB2-
Clear Cell Carcinoma (CCC)	Endometrium	~ 10%	Intermediate: presents at younger age and lower stage. Aggressive.	TP53wt ARID1A high mut PIK3CA high mut PTEN loss HNF1B ubiquitous BRCA1/2 negligible mut	NapsinA+ WT1- p53- ER- CALB2-
Endometrioid Carcinoma (EC)	Endometrium	~ 10%	Favorable: presents at younger age then HGSC, associated with endometriosis.	TP53mut (rare) ARID1A high mut PIK3CA moderate mut CTNNB1 moderate mut PTEN moderate mut BRCA1/2 negligible mut	CK7+ PAX8+ WT1- CK20- FOXL2- CALB2-
Low-Grade Serous Carcinoma (HGSC)	Fallopian Tube Epithelium	< 5%	Intermediate: presents at younger age then HGSC.	TP53 wt RAS-pathway mut BRCA1/2 mut low	CK7+ WT1+ ER+
Mucinous Carcinoma (MC)	Unknown	< 5%	Good: Presents at younger age then HGSC.	TP53 mut moderate KRAS mut high ERBB2 amplification BRCA1/2 mut negligible	CK7+ CK20- ER- PR- WT1-

Figure 2. Schematic of epithelial ovarian carcinoma subtypes.

## Prognostic genes in ovarian cancer

The Human Protein Atlas (HPA) has classified the genes associated with a unfavorable and favorable prognosis in ovarian cancers.

The transcriptome analysis of the ovarian cancer proteome shows that 72% (n= 14467) of all human genes (n=20090) are expressed in ovarian cancer. According to the Pathology section of HPA, in ovarian cancer there are:

- **152 unfavorable genes**
- **358 favorable genes**

For unfavorable genes, higher relative expression levels at diagnosis give significantly lower overall survival for the patients.

For favorable genes, higher relative expression levels at diagnosis give significantly higher overall survival for the patients.

## Examples of prognostic, diagnostic and metastatic markers

**L1CAM:** unfavorable prognostic significance in ovarian cancer (figure 3).

Overexpression of the L1-cell adhesion molecule (L1CAM) has been observed for various carcinomas and correlates with poor prognosis and late-stage disease. L1CAM has emerged as a causal factor in tumor invasion and metastasis.

With reference to ovarian cancers, L1CAM expression contributes to the invasive and metastatic phenotype of high-grade serous ovarian carcinoma (Bondong 2012).

In the study by Abdel Azim (2016), L1CAM expression on the transcriptome level was assessed with quantitative real-time PCR (qRT-PCR) to define its relevance in ovarian cancer biology. The study included fresh frozen tissue samples of 138 FIGO I-IV stage ovarian cancer patients. The results showed that L1CAM expression levels play a substantial role in ovarian cancers pathophysiology, which is translated into poor clinical outcome.

**CD44:** unfavorable prognostic in ovarian cancer progression and metastasis (figure 3).

The Cluster of Differentiation 44 (CD44) is a cell surface adhesion receptor highly expressed in many cancers and regulates metastasis via its recruitment on the cell surface.

Both the metastatic and recurrent ovarian cancer tissues express higher levels of CD44. However, over-expression of CD44 has been mainly found in ovarian epithelial carcinomas, where high levels correlate with poor prognosis and more advanced disease stages (Afify 2001; Cho 2006). A recent systematic meta-analysis of 18 studies with more than 2000 ovarian cancer patients showed a significant correlation between CD44 expression and poor 5-year overall survival, suggesting that CD44 levels are an effective marker for diagnosis and prediction of clinical outcomes in ovarian cancers (Gao 2015; Lin 2017).

It has been hypothesized that stem cell transformation can be the underlying cause of ovarian cancer malignancy. In support of this hypothesis, Bapat et al. (2005) showed that CD44-positive (CD44+) ovarian tumor cells could express stem cell markers, thus initiating tumorigenesis and

promoting disease recurrence by recapitulating the original tumor.

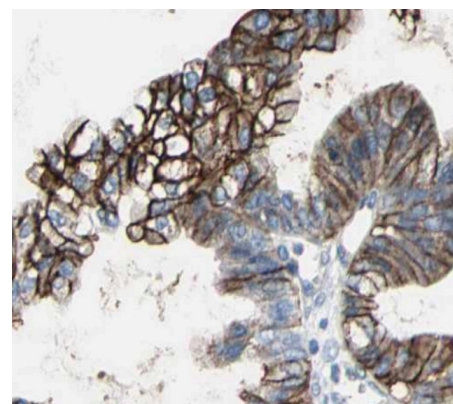
**CLDN3:** diagnostic and prognostic potential (figure 3).

Claudins are a family of proteins representing the essential component of the tight junction. Some claudins are highly elevated in various human cancers, including ovarian cancer.

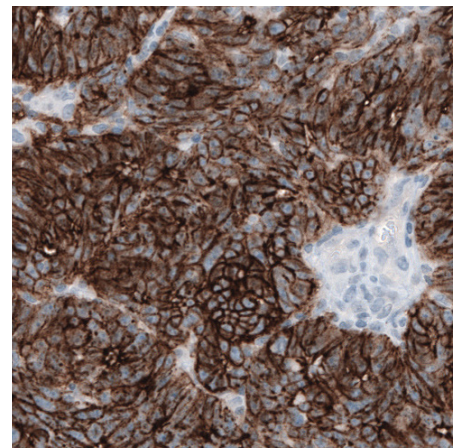
Claudin 3 (CLDN3) is a commonly upregulated gene in 90% of ovarian cancers and is considered an effective marker for early detection (Choi 2007; Uthayanan 2022).

Due to the difficulties in screening for claudins in serum, their assessment by IHC analysis of tumor samples shows promising potential as diagnostic and prognostic biomarkers for ovarian cancers.

**Anti-CD44 (HPA005785)**  
Endometrioid Carcinoma



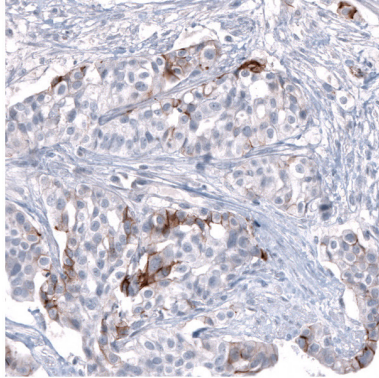
**Anti-CLDN3 (AMAb91834)**  
Endometrioid Carcinoma



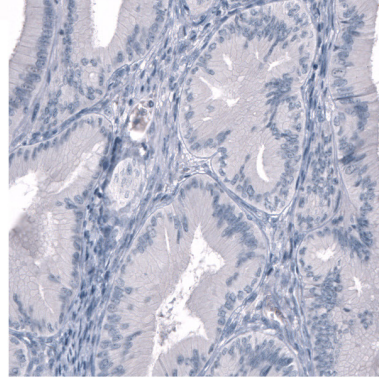
# Prognostic markers for ovarian cancers

## Anti-L1CAM (AMAb91829)

Ovarian Ca, HGSC

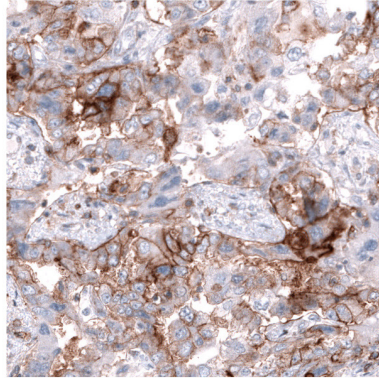


Ovarian Ca, LGSC

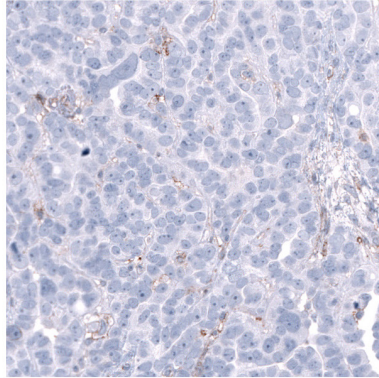


## Anti-CD44 (AMAb91847)

Ovarian Ca, endometrioid

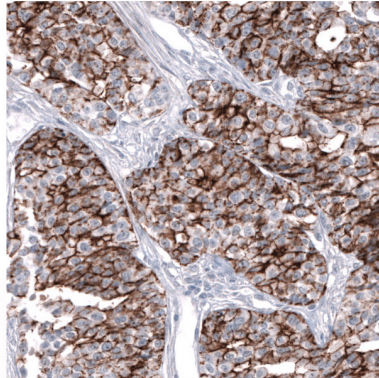


Ovarian Ca, papillary

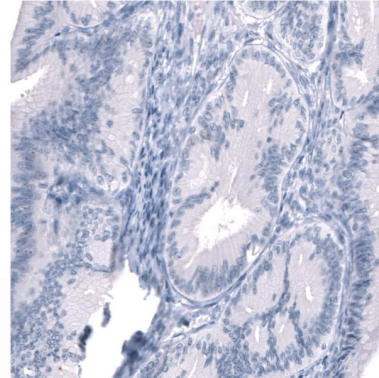


## Anti-CLDN3 (AMAb91835)

Ovarian Ca, HGSC



Ovarian Ca, LGSC



**Figure 3. Prognostic markers for ovarian cancers.** Representative immunohistochemical staining of human female carcinomas using the Anti-L1CAM (AMAb91829), Anti-CD44 (AMAb91847) and Anti-CLDN3 (AMAb91835) PrecisA Monoclonal antibodies showing strong to moderate positivity in tumor cells of the specific ovarian carcinoma subtype (in brown).

## Diagnostic markers

Ovarian cancers are often diagnosed at an advanced stage, therefore treatment outcome is usually poor. Markers that permit an early diagnosis will substantially impact disease outcome.

Currently, the circulating tumor antigen CA125 is the only clinically used marker. However, testing for CA125 is not specific or sensitive enough to be clinically relevant as a screening tool in the general population, especially in the context of early tumor detection. In addition, at least 20% of ovarian tumors fail to express CA125.

Among the new markers with high potential use as diagnostic tools, WT1, NAPSIN A, HE4 (WFDC2), FOXL2 and CALB2 have already demonstrated clinical results.

**WT1: not only a diagnostic but also a prognostic marker in high-grade serous ovarian carcinoma (figure 4).**

Wilms tumor protein 1 (WT1) is a tumor suppressor gene. The prognostic significance of WT1 in patients with advanced serous epithelial ovarian carcinoma has been reported by Netinatsunthorn et al, (2006) who analyzed the immunohistochemical expression of WT1 in tissue from 163 patients diagnosed with advanced serous epithelial ovarian carcinoma.

The results suggested that WT1 overexpression maybe indicative of an unfavorable prognosis in patients with advanced serous epithelial ovarian carcinoma (Netinatsunthorn 2006).

Supporting these results, a recent study by Taube et al. (2016) measured WT1 protein expression by immunohistochemistry in a cohort of 207 primary high-grade serous ovarian carcinomas.

The results show that WT1 expression was a significant favorable prognostic factor in primary high-grade serous ovarian carcinoma regarding overall survival and progression-free survival, which was independent of age, stage, and residual tumor.

The specificity of WT1 for high-grade serous ovarian carcinoma is also supported by data showing that other epithelial ovarian carcinomas, such as mucinous carcinoma, do not express WT1 (Goldstein 2001; Shimizu 2000).

**Napsin A: a specific marker for ovarian clear cell carcinoma (figure 4).**

Ovarian clear cell carcinoma (CCC) is divergent from other types of ovarian epithelial carcinoma in terms of clinicopathologic and molecular features.

NAPSIN A (Napsin A aspartic peptidase) is frequently expressed in CCC of the ovary and endometrium and is a useful immunohistochemical marker to distinguish with high sensitivity and specificity ovarian clear cell carcinoma from high-grade serous and borderline tumors (Alshenavay 2018; Iwamoto 2015; Rekhi 2018)

**FOXL2 and CALB2: sensitive and specific marker for sex cord-stromal tumors of the ovary (figure 4).**

Sex cord-stromal tumors (SCSTs) of the ovary are relatively uncommon tumors. Together with  $\alpha$ -inhibin and calretinin (CALB2), FOXL2 forms an immunomarker panel in essentially SCST.

The immunoeexpression of FOXL2 tested in 501 ovarian tumor samples, including 119 SCSTs shows that FOXL2 staining is present in almost all SCSTs with a FOXL2 mutation, and also in a majority of SCSTs without a mutation (Al-Agha 2011).

Immunohistochemical staining for calretinin (CALB2) is useful in the diagnosis of ovarian sex cord-stromal tumours however, it should always be used as part of a larger panel (McCluggage 2001).

**WFDC2: a diagnostic biomarker for ovarian and endometrial cancer (figure 5).**

Human epididymis protein 4 (HE4) belongs to the family of whey acidic four-disulfide core (WFDC) proteins.

WFDC2/HE4 is overexpressed in 100% of endometrioid, 93% of serous, and 50% of clear cell ovarian carcinomas. In contrast, mucinous or germ cell tumors ovarian carcinomas rarely express it.

In a study of over 200 patients with a pelvic mass, including 67 with epithelial ovarian cancer, WFDC2/HE4 had a higher sensitivity for ovarian cancer detection compared to the cancer antigen 125 (CA125), 72.9% versus 43.3%, respectively (Ferraro 2013).

Moreover, WFDC2/HE4 has an advantage over CA125 because it is less frequently positive in patients with the nonmalignant disease. Researchers also found WFDC2/HE4 to be elevated in more than half of the ovarian cancer patients who did not have elevated CA125 levels; therefore, the combination of WFDC/HE4 and CA125 markers provided slightly improved cancer diagnostic sensitivity for the detection of ovarian cancer (Hellström 2003).

Other useful markers for the diagnosis and prognosis of ovarian cancers include MCR2, CLDN16 KRT7 (figure 5).

# Diagnostic markers for ovarian cancers

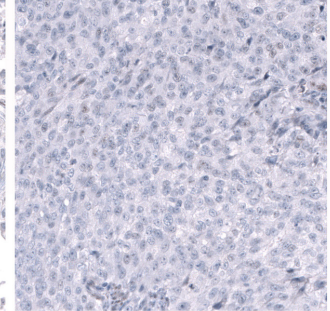
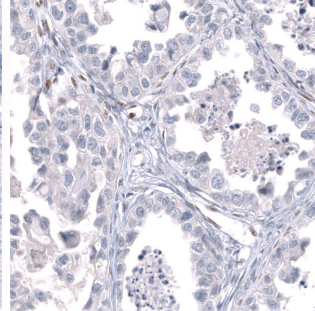
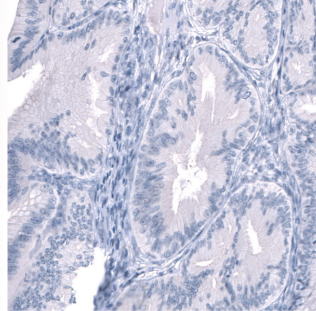
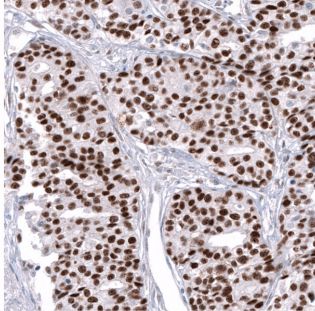
## Anti-WT1 (AMAb91842)

Ovarian Ca, HGSC

Ovarian Ca, LGSC

Ovarian Ca, CCC

Endometrial Ca



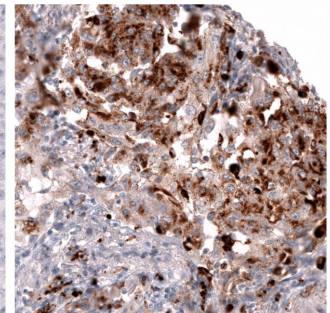
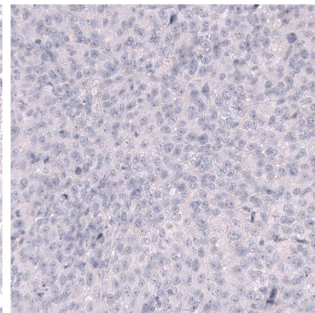
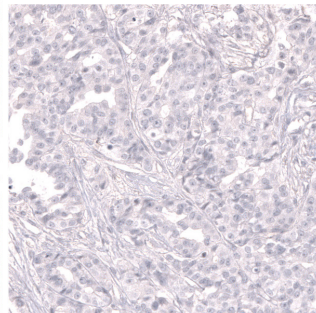
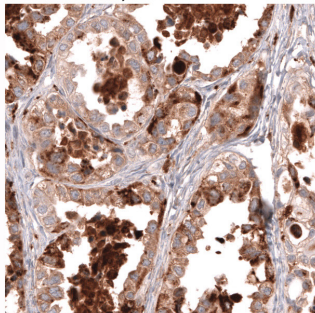
## Anti-NAPSA (AMAb91825)

Ovarian Ca, CCC

Ovarian Ca, HGSC

Endometrial Ca

Lung Ca, Adenocarcinoma



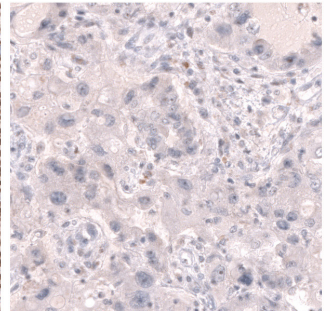
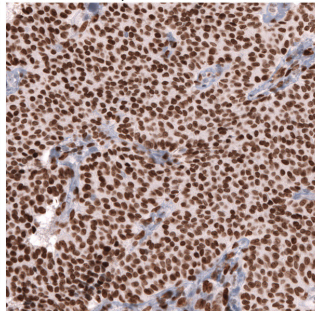
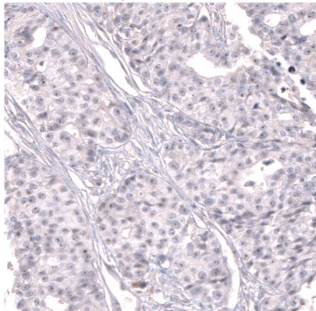
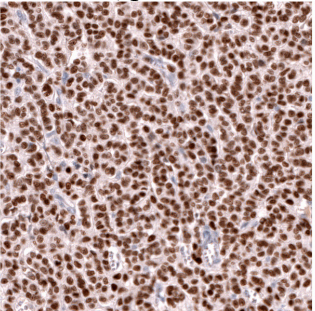
## Anti-FOXL2 (AMAb91808)

Ovarian Ca, granulosa tumor

Ovarian Ca, HGSC

Ovarian Ca, Sex cord tumor

Ovarian Ca, endometrioid



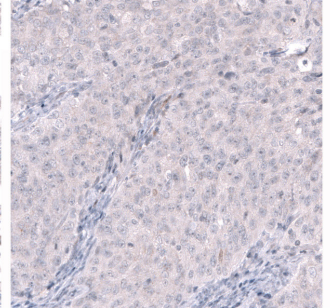
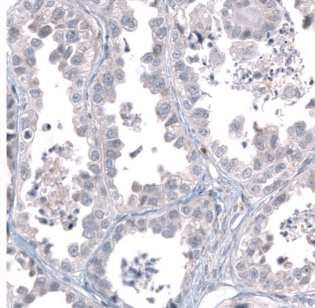
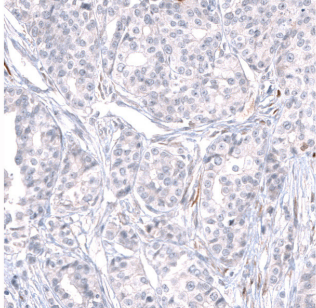
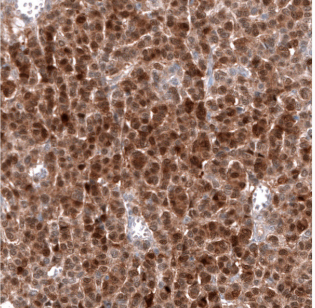
## Anti-CALB2 (AMAb91812)

Ovarian Ca, granulosa tumor

Ovarian Ca, HGSC

Ovarian Ca, CCC

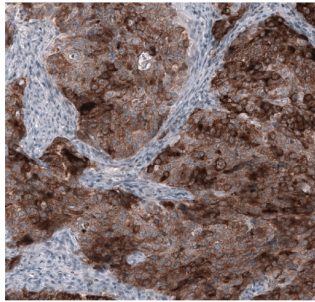
Endometrial Ca



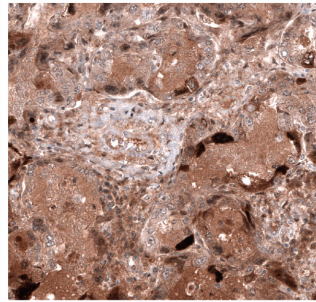
**Figure 4. Diagnostic markers for ovarian cancers.** Representative immunohistochemical staining of human female carcinomas using the Anti-WT1 (AMAb91842), Anti-NAPSA (AMAb91825), Anti-FOXL2 (AMAb91808) and Anti-CALB2 (AMAb91812) PrecisA Monoclonal antibodies showing strong to moderate positivity in tumor cells of the specific ovarian carcinoma subtype (in brown). For NAPSA positive staining is also observed in lung adenocarcinoma (as expected).



**Anti-WFDC2 (AMAb91821)**  
Endometrioid



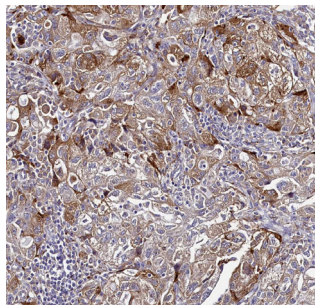
Endometrial



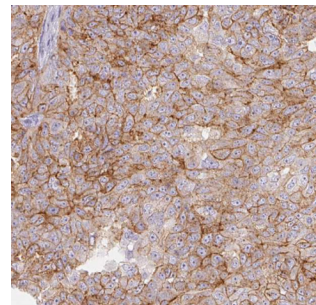
**Figure 5. Markers for ovarian cancers.**

Representative immunohistochemical stainings of ovarian carcinomas showing strong to moderate positivity in tumor cells of the specific ovarian carcinoma subtype (in brown).

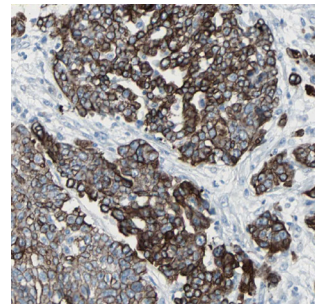
**Anti-MRC2 (HPA041991)**  
Serous, OvCa, Unfavorable



**Anti-CLDN16 (HPA056020)**  
Endometrial, Unfavorable



**Anti-KRT7 (HPA007272)**  
Serous, OvCa



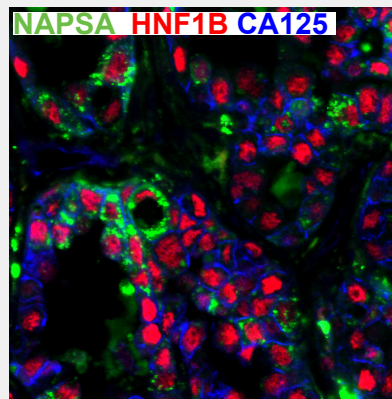
## Multiplexed IHC in gynecological pathology

Immunohistochemical findings play a crucial role in the differential diagnosis of gynecologic carcinomas. Tumors often show aberrant expression of protein type; therefore, using a panel of antibodies is generally recommended.

Multiplexing IHC helps to support a diagnosis of a variety of problematic lesions seen in gynecologic pathology. However, as in any other system, immunohistochemical findings need to be interpreted in light of the clinical history and morphologic findings.

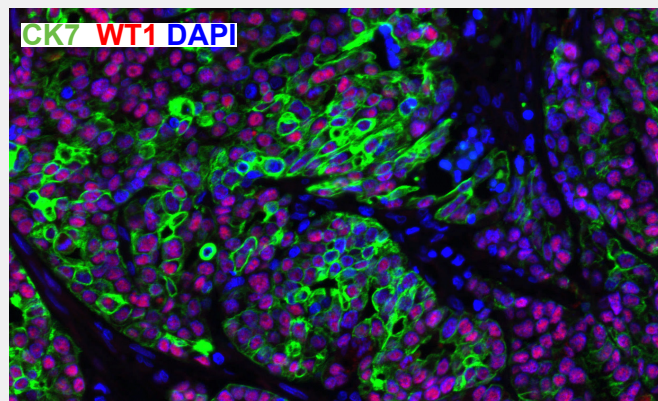
### *OvCa, High Grade Serous Carcinoma*

Multiplexing IHC using the Anti-CK7 (KRT7, AMAb91531, IgG1, green) and Anti-WT1 (AMAb91840, IgG2a, red) useful as markers in diagnosis of HGSC (40x).



### *OvCa, Clear Cell Carcinoma*

Multiplexing IHC using the Anti-NAPSA (AMAb91825, IgG1, green), Anti-HNF1B (HPA002083, pAb, red) and Anti-CA-125 (MUC16, AMAb91057, IgG2b, blue) antibodies as useful markers in the diagnosis of clear cell ovarian carcinoma (40x).



## Metastatic markers

The value of **CDX2** as a metastatic marker (figure 6).

CDX2 is a homeobox protein responsible for the maintenance of the intestinal phenotype. Positive CDX2 staining predicts metastasis from a midgut or hindgut origin gastrointestinal carcinoid.

The value of CDX2 in detecting colonic carcinoma metastatic to the ovary has been revealed in a study by Groisman et al, (2004) who evaluated CDX2, CK7, and CK20 expression by IHC in 50 ovarian carcinomas (15 serous, 20 mucinous, and 15 endometrioid), 15 colonic carcinomas metastatic to the ovaries, and 20 primary colonic carcinomas.

The results show that CDX2 is a highly sensitive (100%) marker for colonic carcinoma metastatic to the ovary and is more specific than CK20 because it is not expressed by serous, mucinous, and endometrioid carcinomas.

Moreover, Vang et al. (2006) found that, as a single marker, CDX2 offers some advantage over other markers (such as cytokeratin 20), being 40% less frequently expressed in primary ovarian tumors compared to cytokeratin 20 (83%).

The lack of an anatomic barrier allows ovarian cancer cells to spread into the peritoneal cavity. Carcinomas that most commonly metastasize to the ovary include those from the endometrium, colon, and breast.

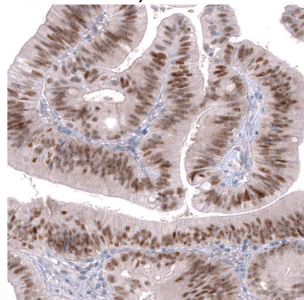
As a result, the histologic differentiation of primary ovarian carcinoma from other metastatic carcinomas to the ovaries may be difficult.

Even though metastasis is the leading cause of ovarian cancer-related fatalities, our understanding of the process remains limited.

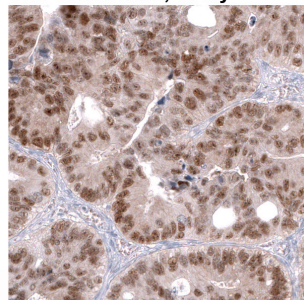
Table 1, adapted from Khush Mittal et al. (2008), suggests a panel of antibodies for each differential diagnosis. These panels may be modified based on the user's personal experience and the antibodies' local availability.

### Anti-CDX2 (AMAb91828)

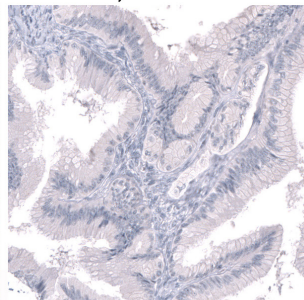
Colorectal Ca, CRC



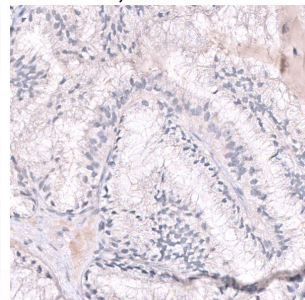
CRC metastasis, ovary



Ovarian Ca, LGSC



Ovarian Ca, mucinous



**Figure 6.** Representative immunohistochemical staining of colorectal carcinoma metastasis to the ovary and ovarian carcinomas using the Anti-CDX2 (AMAb91828) PrecisA Monoclonal antibody showing strong to moderate positivity in metastatic tumor cells (in brown). Primary colorectal tumor also display nuclear positivity (as expected).

**Table 1. Suggested markers panel to distinguish primary ovarian carcinomas from NON-ovarian metastatic carcinomas to the ovaries.**

Primary Ovarian Carcinomas	Non-Ovarian Metastatic Carcinomas	Suggested Markers Panel
Primary Ovarian Adenocarcinoma	Adenocarcinomas Metastatic to the Ovary	CK7, CK20, WT1, ER, PR, GCDFP-15, CDX2, DPC4, p16, $\beta$ -catenin
Primary Endometrioid Carcinoma	Metastatic Colon Carcinoma	CK7, CK20, ER, PR, CA 125
Primary Mucinous Carcinoma	Metastatic Colon Carcinoma	CK7, CA 125, ER, PR, CDX2, MUC5AC
Primary Clear Cell Carcinoma	Metastatic Renal Clear Cell Carcinoma	CK7, CA 125, CD10, ER, PR
Ovarian Adenocarcinoma	Metastatic Breast Carcinoma	GCDFP-15, vimentin, ER, PR
Ovarian Adenocarcinoma	Metastatic Pancreatic/Bile Duct Carcinoma	CA 19-9, DPC4, ER, PR
Ovarian Adenocarcinoma	Adenocarcinoid	chromogranin, CD56
Ovarian Adenocarcinoma	Sex Cord Stromal Tumor	inhibin, Ber-EP4, EMA, AE1/AE3

# Enhanced Validation: an additional layer of security in antibody validation



At Atlas Antibodies, we take great care to validate our antibodies in IHC, WB, and ICC-IF. Enhanced Validation is performed as an additional layer of security in an application and context-specific manner.

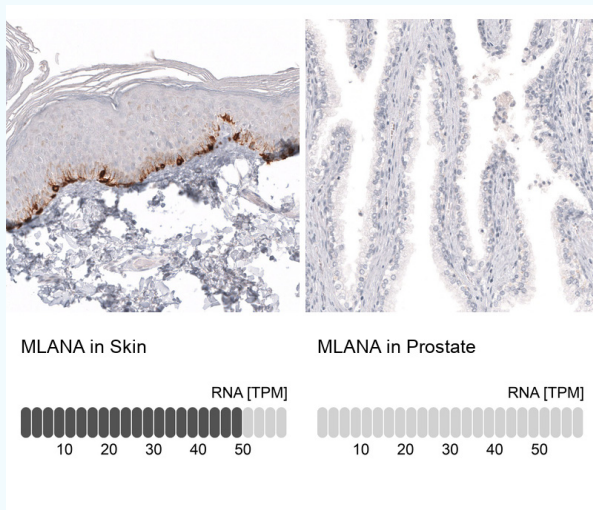
Enhanced Validation follows the guidelines proposed by the International Working Group for Antibody Validation (IWGAV) and published in Nature Methods\*. By having all five methods recommended by IWGAV at our disposal, we have the power to validate a wide range of different antibodies.

\* Uhlen, M., Bandrowski, A., Carr, S. et al. A proposal for validation of antibodies. *Nat Methods* 13, 823–827 (2016).

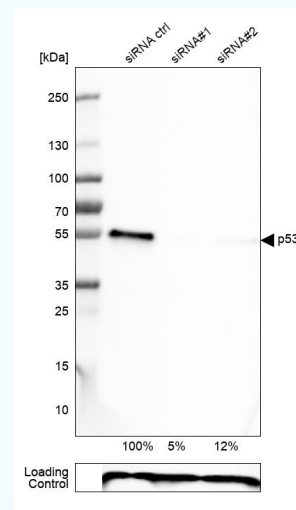
Enhanced validation offers increased security of antibody specificity in a defined context. By using 5 different enhanced validation methods we validate our antibodies for each combination of protein, sample, and application.

The 5 methods are:

- Genetic validation,
- Orthogonal validation,
- Validation by independent antibodies,
- Recombinant expression validation,
- Migration capture MS validation.



Example of **orthogonal validation in IHC** of protein expression using IHC by comparison of the staining signal to the RNA-seq data (TPM) of corresponding target in high and low expression tissues. The image shows the immunohistochemistry analysis in human skin and prostate tissues using the Anti-MLANA (AMAb91817) PrecisA Monoclonal antibody. Corresponding MLANA RNA-seq data (TPM) are presented for the same tissues.



Example of **genetic validation in WB** by siRNA knockdown. The image shows the Western blot analysis in U-251MG cells transfected with control siRNA, target specific siRNA probe #1 and #2, using the Anti-p53 (AMAb90956) PrecisA monoclonal antibody. Remaining relative intensity is presented. Loading control: Anti-PPIB.

## PrecisA Monoclonals targeting ovarian cancers.

We are continuously updating our catalogs. Please refer to the online version for the latest updates of this document. Check our website to discover related products, such as TripleA Polyclonals™ and PrEST Antigens™, for each antibody listed in this table.

Product Name	Protein Name	Product Number	Isotype	Validated Applications
Anti-CALB2	Calbindin 2	AMAb91812	IgG1	IHC*, WB*
		AMAb91813	IgG2a	IHC*
		AMAb91814	IgG1	IHC*, WB*
Anti-CD44	CD44 antigen	AMAb91845	IgG3	IHC*, WB*
		AMAb91846	IgG2a	IHC*, WB*
		AMAb91847	IgG2b	IHC*, WB*
		AMAb91848	IgG1	IHC*, WB*
Anti-CD99 (MIC2)	Cd99 molecule (xg blood group)	AMAb91823	IgG2a	IHC
Anti-CDKN1A (p21)	Cyclin-dependent kinase inhibitor 1	AMAb91832	IgG1	WB, ICC-IF
Anti-CDX2	Caudal type homeobox 2	AMAb91827	IgG1	IHC*, WB*, ICC-IF
		AMAb91828	IgG2b	IHC*, WB*, ICC-IF
Anti-CLDN3	Claudin-3	AMAb91834	IgG1	IHC*, WB*, ICC-IF
		AMAb91835	IgG2a	IHC*, WB*, ICC-IF
		AMAb91836	IgG2b	IHC*, WB*, ICC-IF
Anti-FOXL2	Forkhead box protein L2	AMAb91808	IgG3	IHC*, WB
		AMAb91809	IgG1	IHC*
Anti-FOXO1	Forkhead box protein O1	AMAb91844	IgG1	WB*
Anti-GPC3	Glypican 3	AMAb91803	IgG2b	IHC*
Anti-GSPT1	Euk pep chain fact GTP-binding ERF3A	AMAb91849	IgG2a	IHC, WB
		AMAb91851	IgG2a	IHC, WB, ICC-IF
Anti-L1CAM (CD171)	Neural cell adhesion molecule L1	AMAb91829	IgG2a	IHC*
		AMAb91830	IgG1	IHC*
Anti-MCAM (CD146)	Cell surface glycoprotein MUC18	AMAb91815	IgG1	IHC*
Anti-MLANA (MART1)	Melanoma antigen	AMAb91816	IgG1	IHC*, WB*, ICC-IF
		AMAb91817	IgG1	IHC*, WB*, ICC-IF
		AMAb91818	IgG1	IHC*, WB*, ICC-IF
Anti-NAPSA	Napsin-A aspartic peptidase	AMAb91825	IgG1	IHC*
Anti-NCAM1 (CD56)	Neural cell adhesion molecule 1	AMAb91807	IgG2b	IHC*
Anti-TFE3	Transcription factor E3	AMAb91798	IgG2b	IHC, WB
		AMAb91799	IgG1	IHC, WB, ICC-IF
Anti-TNFRSF8 (CD30)	TNF receptor superfamily member 8	AMAb91800	IgG2a	IHC
Anti-WFDC2 (HE4)	Wap four-disulfide core domain 2	AMAb91819	IgG1	IHC*
		AMAb91821	IgG1	IHC*
Anti-WT1	Wilms tumor protein	AMAb91839	IgG2b	IHC*, WB*, ICC-IF
		AMAb91840	IgG2a	IHC*, WB*, ICC-IF
		AMAb91842	IgG1	IHC*, WB*, ICC-IF

\* Enhanced Validation

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