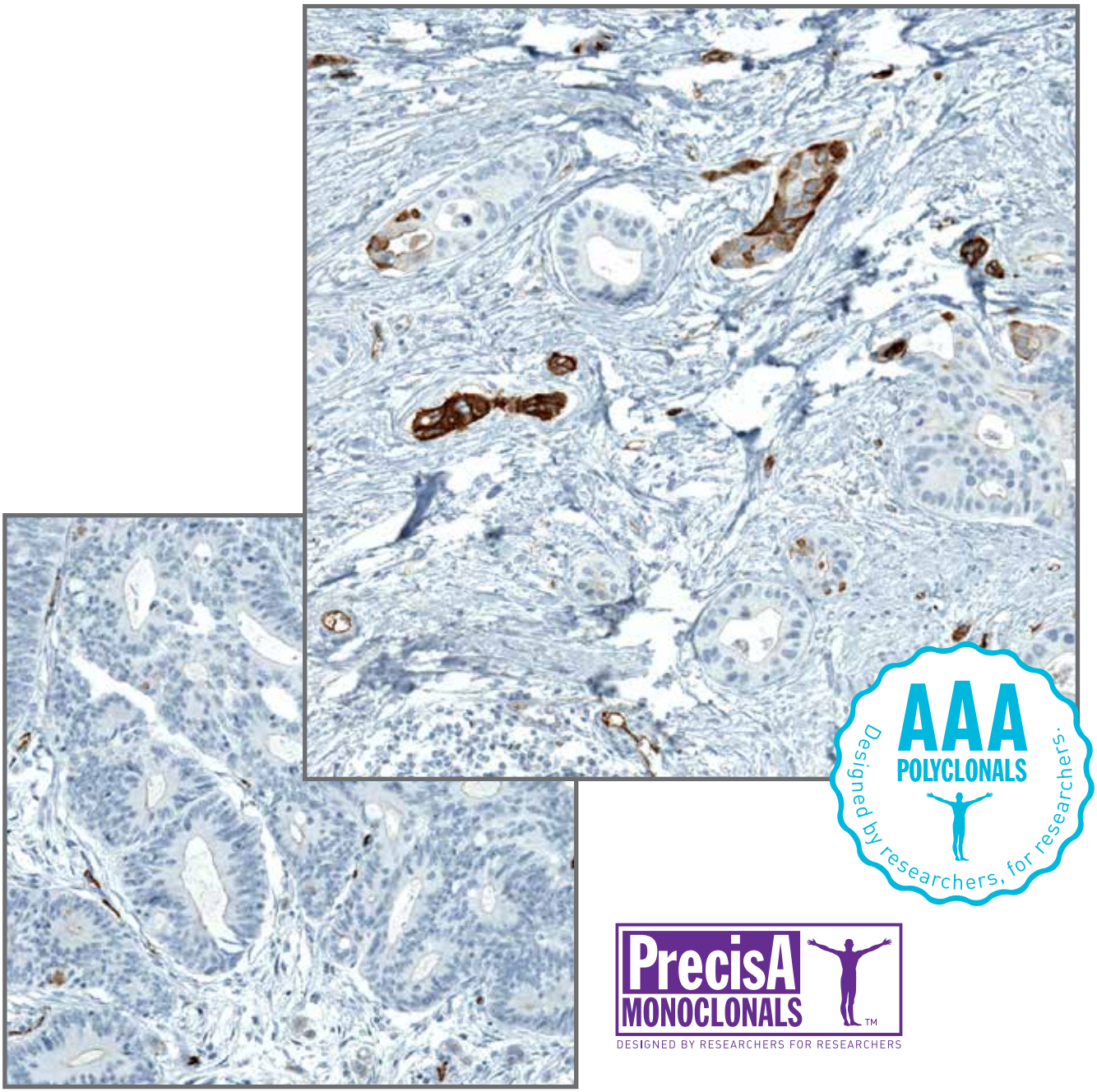

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Oncology Biomarkers

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Oncology Biomarkers

Need for Novel Cancer Biomarkers

The microscopic evaluation of stained tissue sections from a tumor remains the gold standard for cancer diagnosis. However, in order to optimize patient treatment and provide guidance for therapeutic intervention of the underlying disease there is often a need for additional tumor stratification methods.

The analysis of protein expression in cells from a tumor tissue often provides important additional information to the pathologist. Immunohistochemistry (IHC) using protein specific antibodies provides a tool to detect the presence, abundance and localization of specific proteins.

With a limited repertoire of protein biomarkers available today there is a clear and unmet clinical need to identify novel sets of biomarkers. The aim is to provide a more accurate diagnosis and a better assessment of patient prognosis, ultimately leading to a more individualized treatment.

Atlas Antibodies recognize this need for new biomarkers. Together with our research partners in the Human Protein Atlas project we are in a unique position to perform antibody-based biomarker discovery.

Biomarkers for Licensing

Several proprietary oncology biomarkers are available for licencing from Atlas Antibodies, both Triple A Polyclonals and PrecisA Monoclonals. A selection of available antibodies is presented below.

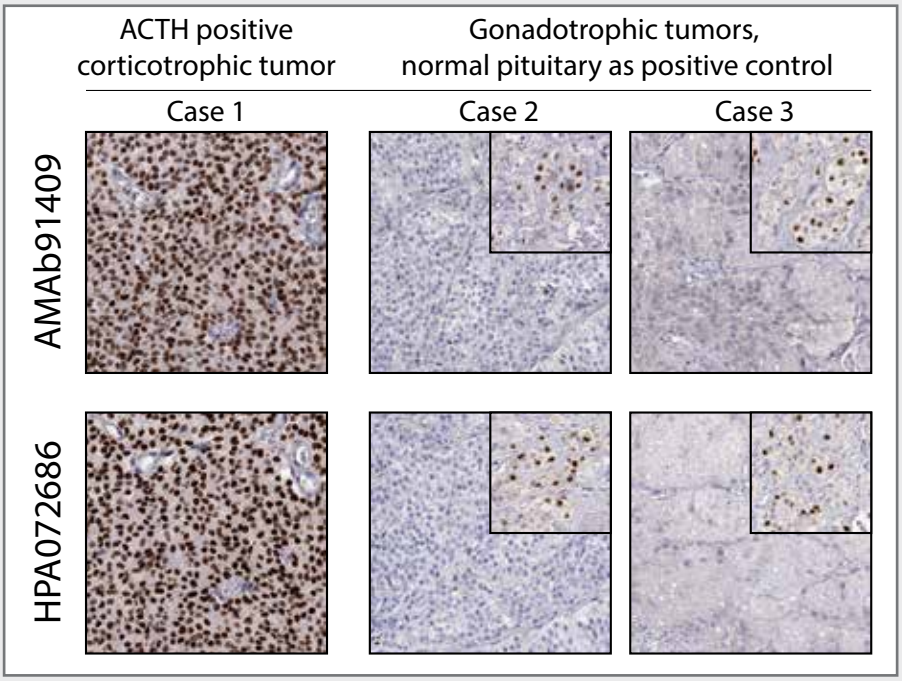
Additional information on licensing of specific biomarkers and information about Atlas Antibodies' customized solutions and validation capabilities is available on request by sending an email to: *contact@atlasantibodies.com* or visit our Research Collaborations page (*atlasantibodies.com/research/collaborations*).

T-PIT - A tool for classification of pituitary neuroendocrine tumors

Non-functioning pituitary neuroendocrine tumors (NF-PitNETs) is a heterogeneous group of hypophyseal neoplasms characterized by the lack of hormone hypersecretion. Absence of endocrine symptoms makes the diagnosis and adequate treatment of these tumors difficult. The use of pituitary-specific transcription factors has therefore been proposed for more precise classification of NF-PitNETs[2,3].

Three major transcription factors determining cell lineages are present in the adenohypophysis, including Pit-1 (POU1F1), SF-1 (NR5A1) and T-Pit (TBX19). Importantly, expression of these transcription factors is preserved in both primary tumors and metastases.

T-Pit (TBX19) regulates development and differentiation of corticotroph ACTH-producing cells in the adenohypophysis[1]. In a recent study on a patient cohort with different types of pituitary adenomas[4], distinct T-Pit immunoreactivity was demonstrated using the Anti-T-Pit antibody HPA072686. T-Pit immunoreactivity was detected in the ACTH-expressing corticotroph cells in the normal pituitary and in all the corticotroph tumors, both silent and functioning.



Immunohistochemical staining of ACTH-positive corticotrophic tumor (**Case 1**) and gonadotrophic tumors (**Case 2 and 3**) using Anti-T-Pit monoclonal (AMAb91409, upper panels) and polyclonal (HPA072686, lower panels) antibodies. Note strong nuclear positivity in the majority of cells in corticotrophic tumor and absence of immunoreactivity in gonadotroph tumors. Insets in Case 2 and 3 images show nuclear positivity in normal adenohypophysis. Identical staining pattern is observed with both monoclonal and polyclonal antibody.

Moreover, T-Pit expression was detected in 8 of 15 tumors previously classified as null-cell adenomas, thus increasing the diagnostic accuracy.

Monitoring of T-Pit thus allows for a more precise classification and better identification of NF-PitNETs and may contribute to adequate and timely treatment of patients.

References

1. Lamolet B *et al.* A pituitary cell-restricted T box factor, Tpit, activates POMC transcription in cooperation with Pitx homeoproteins. *Cell.* 2001 Mar 23;104(6):849-59.

2. Lloyd SKW *et al.* Hearing optimisation in neurofibromatosis type 2: A systematic review.
Clin Otolaryngol. 2017 Dec;42(6):1329-1337

3. Manojlovic-Gacic E *et al. Histopathological classification of non-functioning pituitary neuroendocrine tumors.*
Pituitary. 2018 Apr;21(2):119-129.

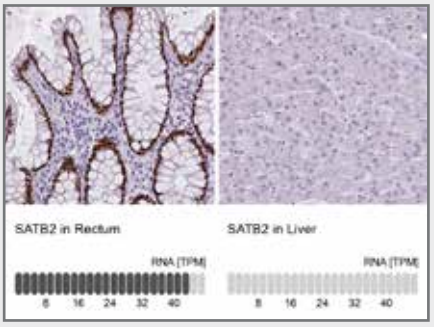
4. Sjöstedt E *et al.* A specific antibody to detect transcription factor T-Pit: a reliable marker of corticotroph cell differentiation and a tool to improve the classification of pituitary neuroendocrine tumours. *Acta Neuropathol,* 2017 Oct; 134(4):675-677.

SATB2 – A diagnostic biomarker for tumors of colorectal origin

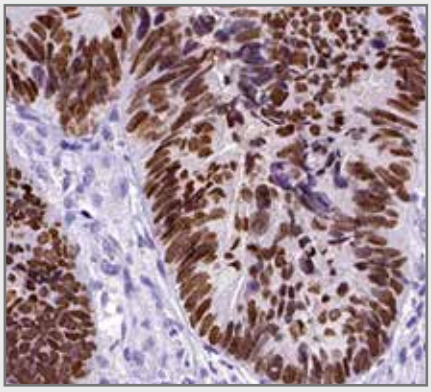
Cell- and cancer-type specific proteins are rare. The special AT-rich sequence-binding protein SATB2 has been identified as having a very selective expression pattern. In cells of epithelial lineages, SATB2 is expressed in glandular cells lining the lower gastrointestinal tract and expression is retained in a large majority of primary and metastatic colorectal cancers. Thus, SATB2 is a promising diagnostic biomarker for tumors of colorectal origin.

In a previously published study by Magnusson *et al.* it was shown, by analyzing more than 1,800 tumor samples, that SATB2 expression is largely preserved in cells of colorectal cancer origin. More than 85% of all colorectal cancers showed distinct SATB2 immunostaining and when used in combination with Cytokeratin 20 analysis, SATB2 identified more than 95% of all tumors with colorectal origin.

These promising data suggested that the combination of SATB2 and CK20 should be tested in an unbiased clinical study to further validate the initial findings. In a recent publication by Dragomir et al, the expression of SATB2 was analyzed in over 800 consecutive clinical cases for which CK20 immunostaining was considered necessary to obtain a final diagnosis. In this study, SATB2 showed 93% sensitivity and 77% specificity to determine a cancer of colorectal origin, and in combination with CK7 and CK20, the specificity increased to 100%. SATB2 thus provides a new and advantageous supplement to current standards for clinical differential diagnosis.



Orthogonal validation:
Immunohistochemistry analysis in human rectum and liver tissues using AMAb90679 antibody. Corresponding SATB2 RNA-seq data are presented for the same tissues.



Immunohistochemical staining of human colorectal tumor with Anti-SATB2 antibody (HPA029543) shows strong nuclear staining in tumor cells.

Related Publications

Dragomir A *et al.* The role of SATB2 as a diagnostic marker for tumors of colorectal origin: Results of a pathology-based clinical prospective study. *Am J Clin Pathol.* 2014 May;141(5):630-8.

Elebro J *et al.* Prognostic and treatment predictive significance of SATB1 and SATB2 expression in pancreatic and periampullary adenocarcinoma. *J Transl Med* , 2014 Oct 17; 12:289.

Hedner C *et al.* SATB1 is an independent prognostic factor in radically resected upper gastrointestinal tract adenocarcinoma. *Virchows Arch* , 2014 Dec; 465(6):649-659.

Magnusson K *et al.* SATB2 in combination with cytokeratin 20 identifies over 95% of all colorectal carcinomas. *Am J Surg Pathol.* 2011 Jul;35(7):937-48.

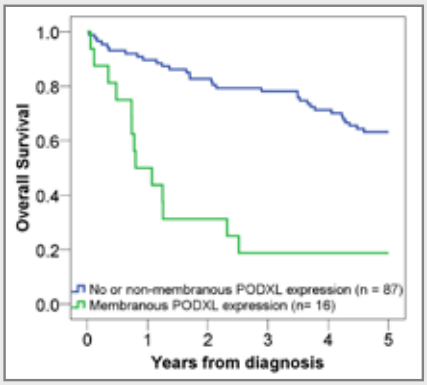
Nodin B *et al.* Molecular correlates and prognostic significance of SATB1 expression in colorectal cancer. *Diagn Pathol,* 2012 Aug 30; 7:115.

PODXL - An independent factor for poor prognosis and treatment stratification

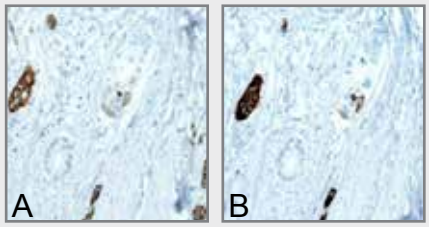
Podocalyxin-like 1 (PODXL) is a cell-adhesion glycoprotein and stem cell marker that has been associated with aggressive tumor phenotype and adverse outcome in several cancer types.

In a number of papers, Larsson *et al.* have demonstrated that membraneous expression of PODXL is associated with unfavourable clinicopathological characteristics and independently predicts a poor prognosis in colorectal cancer (CRC). This has been demonstrated in three independent patient cohorts in total comprising more than 1,000 patients. The results clearly demonstrate the potential utility of PODXL as a biomarker for more precise prognostication and treatment stratification in CRC.

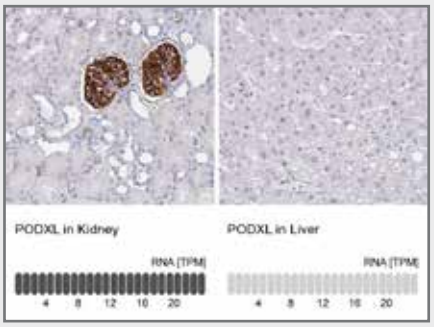
Boman *et al.* have investigated the prognostic impact of membraneous PODXL expression in almost 500 cases of urothelial cancer. They concluded that PODXL is indeed an independent risk factor for progressive disease and death in patients with urothelial cancer and that this warrant further studies to fully evaluate the use of PODXL as a biomarker for improved treatment stratification of bladder cancer patients.



Kaplan–Meier estimates of 5-year Overall Survival (OS) according to PODXL expression in a urothelial cancer patient cohort of 110 individuals.



Immunohistochemical staining of PODXL protein in colorectal tumor tissue using HPA002110 (**A**) and AMAb90667 (**B**) antibodies.



Orthogonal validation:
Immunohistochemistry analysis in human kidney and liver tissues using AMAb90667 antibody. Corresponding PODXL RNA-seq data are presented for the same tissues.

Related Publications

Boman K *et al.* Podocalyxin-like and RNA-binding motif protein 3 are prognostic biomarkers in urothelial bladder cancer: a validatory study. *Biomark Res* , 2017 Mar 14; 5:10.

Boman K *et al.* Membraneous expression of podocalyxin-like protein is an independent factor of poor prognosis in urothelial bladder cancer. *Br J Cancer.* 2013 Jun 11;108(11), 2321-2328.

Borg D *et al.* Expression of podocalyxin-like protein is an independent prognostic biomarker in resected esophageal and gastric adenocarcinoma. *BMC Clin Pathol* , 2016 Jul 29; 16:13.

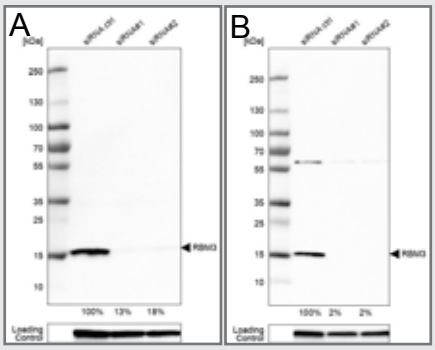
Kusumoto H *et al.* Podocalyxin influences malignant potential by controlling epithelial–mesenchymal transition in lung adenocarcinoma *Cancer Sci* , 2017 Apr 3; 108(3):528-535.

Larsson A *et al.* Overexpression of podocalyxin-like protein is an independent factor of poor prognosis in colorectal cancer. *Br J Cancer* 2011 Aug 23;105(5):666-72.

RBM3 - A prognostic and treatment predictive biomarker

The RNA-binding protein RBM3 has been identified via the Human Protein Atlas as an oncology biomarker through the differential expression pattern observed within several investigated cancers.

In general, the levels of RBM3 expression have been found to have a significant correlation to patient survival in breast, colon, ovarian, testicular, prostate and urothelial cancer as well as in malignant melanoma. The Anti-RBM3 antibodies from Atlas Antibodies has shown excellent specificity in Western blot analysis and is routinely used for staining of formalin fixed paraffin embedded tissues in IHC. The specificity of the antibodies has been confirmed using siRNA knock-down experiments in WB and ICC-IF.



Genetic validation, siRNA knockdown:
Western blot analysis in U-251MG cells transfected with control siRNA, target specific siRNA probe #1 and #2, using Anti-RBM3 antibody AMAb90655 (**A**) and HPA003624 (**B**). Remaining relative intensity is presented. Loading control: Anti-GAPDH.

A major clinical challenge in urothelial cancer is the identification of high-risk patients among those diagnosed with non-invasive disease. Our data suggests that RBM3 expression analysis could be used as a prognostic factor for better stratification of patients, leading to a more individualized treatment of patients with urothelial cancer. The prognostic significance of RBM3 in urothelial cancer has been confirmed in several independent cohorts.

Our findings indicate that patients whose tumors express high levels of RBM3 could benefit from platinum-based treatment, whereas alternative treatments may be considered for patients having no or low RBM3 expression. In vitro cell-line experiments have also demonstrated a role of RBM3 in respect to treatment with platinum-based drugs like cisplatin.

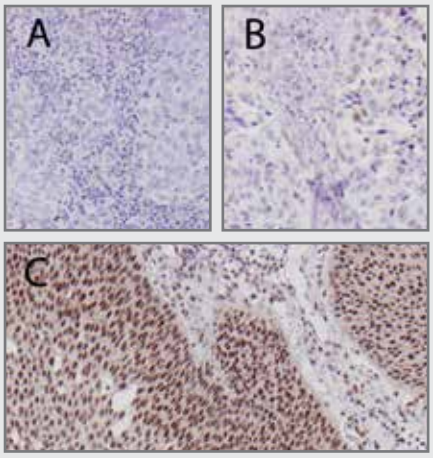
Related Publications

Boman K *et al.* Decreased expression of RNA-binding motif protein 3 correlates with tumour progression and poor prognosis in urothelial bladder cancer.
BMC Urol. 2013, 13:17.

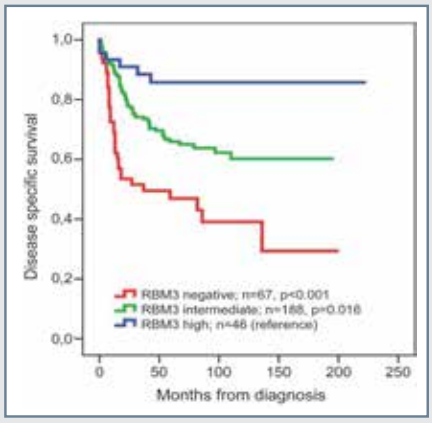
Florianova L *et al.* Evaluation of RNA-binding motif protein 3 expression in urothelial carcinoma of the bladder: an immunohistochemical study.
World J Surg Oncol , 2015 Nov 14; 13(None):317.

Karnevi E *et al.* Translational study reveals a two-faced role of RBM3 in pancreatic cancer and suggests its potential value as a biomarker for improved patient stratification.
Oncotarget , 2017 Dec 15; 9(5):6188-6200.

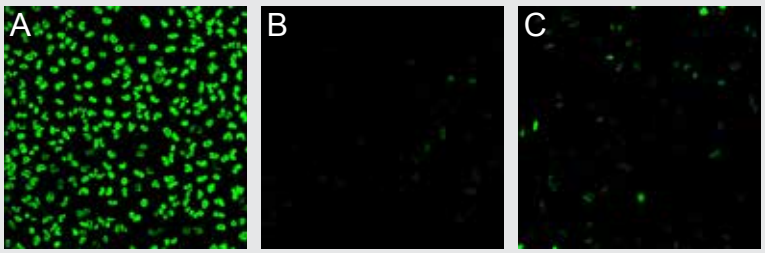
Hjelm B *et al.* High nuclear RBM3 expression is associated with an improved prognosis in colorectal cancer.
Proteomics Clin Appl. 2011 Dec;5(11-12):624-35.



Representative nuclear staining of RBM3 in urothelial cancer, with no RBM3 expression (**A**), intermediate RBM3 expression (**B**) and high RBM3 expression (**C**) respectively. Immunohistochemistry analysis is performed using the Anti-RBM3 antibody AMAb90655.



Survival analysis of urothelial cancer patients stratified according to RBM3 expression.



Genetic validation, siRNA knockdown:
Immunofluorescence staining of A549 cells transfected with control siRNA (**A**), probe 1 (**B**) and probe 2 (**C**), using the Anti-RBM3 monoclonal antibody AMAb90655, showing specific staining of the nucleoplasm in green.

Product Name	Catalog No	Isotype	Application	Sequence Identity Mouse/Rat
Anti-TBX19	AMAb91409	Mouse IgG1 (Monoclonal)	IHC	82%/32%
Anti-TBX19	HPA072686	Rabbit IgG (Polyclonal)	IHC	82%/32%
Anti-SATB2	AMAb90679	Mouse IgG1 (Monoclonal)	IHC, WB	100%/100%
Anti-SATB2	HPA029543	Rabbit IgG (Polyclonal)	IHC*, WB, ICC-IF	100%/100%
Anti-PODXL	AMAb90667	Mouse IgG1 (Monoclonal)	IHC*, WB	43%/41%
Anti-PODXL	HPA002110	Rabbit IgG (Polyclonal)	IHC*, WB, ICC-IF	43%/41%
Anti-RBM3	AMAb90655	Mouse IgG1 (Monoclonal)	IHC*, WB*, ICC-IF	94%/96%
Anti-RBM3	HPA003624	Rabbit IgG (Polyclonal)	IHC, WB*, ICC-IF	94%/96%

* Products with enhanced validation for indicated application

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ATLAS ANTIBODIES

Atlas Antibodies AB
Voltavägen 13A
SE-168 69 Bromma, Sweden
atlasantibodies.com

Phone +46(0)8 54 59 58 50
Fax +46(0)8 54 59 58 51
contact@atlasantibodies.com
support@atlasantibodies.com