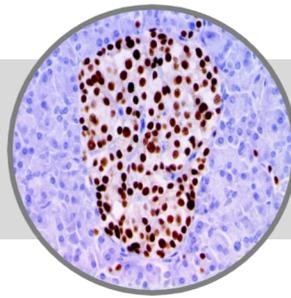


# PAX-6, RMAb

**Clone: EP341**

Rabbit Monoclonal

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*Inset: IHC of PAX-6 on a FFPE Pancreas Tissue*

## Intended Use

For Research Use Only.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

\* The PAX-6 antibody, clone EP341, has been manufactured using Epitomics RabMAb® technology covered under Patent No.'s 5,675,063 and 7,402,409.

## Immunogen

A synthetic peptide corresponding to residues of human PAX6 protein.

## Summary and Explanation

Paired box protein PAX-6 also known as aniridia type II protein (AN2) is a protein that in humans is encoded by the PAX6 gene. PAX6 is a transcription factor present during embryonic development of sensory organs (including eye, nasal and olfactory tissues), central nervous and endocrine system. Within the brain, the protein is involved in development of the specialized cells that process smell. As a transcription factor, PAX6 activates and/or deactivates gene expression patterns to ensure for proper development tissues. Mutations of the PAX6 gene are known to cause various disorders of the eyes. Two common disorders associated with a mutation are: aniridia, the absence of the iris, and Peter's anomaly, thinning and clouding of the cornea.

PAX6 labels neuroendocrine cells and derived tumor cells and is helpful in identification of neuroendocrine tumors. A recent study showed that PAX6 and PAX8 were positive in the majority of neuroendocrine tumors originated from pancreas, duodenum, and colon. Additionally, Neuroendocrine tumors of the lung (NELC), which account for 25% of all lung cancer cases, and transcription factors may drive dedifferentiation of these tumors. SOX4 ( $p = 0.0002$ ), SOX11 ( $p < 0.0001$ ) and PAX6 ( $p = 0.0002$ ) have been found to be significant for tumor type and elevated PAX6 and SOX11 expression correlates with poor outcome in large cell neuroendocrine carcinomas and small cell lung cancer ( $p < 0.0001$  and  $p = 0.0232$ , respectively) based on survival data of 34 patients (57%). Therefore, aggressiveness of NELC correlated with increasing expression of transcription factors.

|                           |                   |                              |  |
|---------------------------|-------------------|------------------------------|--|
| <b>Antibody Type</b>      | Rabbit Monoclonal | <b>Clone</b>                 | EP341                                      |
| <b>Isotype</b>            | IgG               | <b>Reactivity</b>            | Paraffin, Frozen                           |
| <b>Localization</b>       | Nuclear           | <b>Control</b>               | Pancreas, Pituitary, Neuroendocrine Tumors |
| <b>Species Reactivity</b> |                   | Human, Predicted: Mouse, Rat |  |

## Presentation

PAX-6 is a rabbit monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

| <i>Catalog No.</i> | <i>Antibody Type</i> | <i>Dilution</i> | <i>Volume/Qty</i> |
|--------------------|----------------------|-----------------|-------------------|
| BSB 3127           | Tinto Prediluted     | Ready-to-Use    | 3.0 mL            |
| BSB 3128           | Tinto Prediluted     | Ready-to-Use    | 7.0 mL            |
| BSB 3129           | Tinto Prediluted     | Ready-to-Use    | 15.0 mL           |
| BSB 3130           | Concentrated         | 1: 50 - 1: 200  | 0.1 mL            |
| BSB 3131           | Concentrated         | 1: 50 - 1: 200  | 0.5 mL            |
| BSB 3132           | Concentrated         | 1: 50 - 1: 200  | 1.0 mL            |

## Control Slides Available

| <i>Catalog No.</i> | <i>Quantity</i> |
|--------------------|-----------------|
| BSB 3133           | 5 slides        |

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide ( $\text{NaN}_3$ ) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker or similar).
8. For additional safety information refer to Safety Data Sheet for this product.
9. For complete recommendations for handling biological specimens, please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (see References in this document).

**Storage** Store at 2-8°C (Control Slides: Store at 20-25°C)

## Stability

**This product is stable up to the expiration date on the product label.** Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

## Specimen Preparation

**Paraffin sections:** The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

**Frozen sections and cell preparations:** The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

## Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

### a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

### b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

### c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with ImmunoDNA washer or DI water.
9. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

## Abbreviated Immunohistochemical Protocol

| Step                     | ImmunoDetector AP/HRP | PolyDetector AP/HRP | PolyDetector Plus HRP |
|--------------------------|-----------------------|---------------------|-----------------------|
| Peroxidase/AP Blocker    | 5 min.                | 5 min.              | 5 min                 |
| Primary Antibody         | 30-60 min.            | 30-60 min.          | 30-60 min.            |
| 1st Step Detection       | 10 min.               | 30-45 min.          | 15 min.               |
| 2nd Step Detection       | 10 min.               | Not Applicable      | 15 min.               |
| Substrate-Chromogen      | 5-10 min.             | 5-10 min.           | 5-10 min.             |
| Counterstain / Coverslip | Varies                | Varies              | Varies                |

## Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (BSB 0094-0097), refer to PI0174 or PI0097.

## Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a qualified medical professional.

## References

1. Jordan T, et al. The human PAX6 gene is mutated in two patients with aniridia. *Nat. Genet.* 1992; 1 (5): 328-32.
2. Lai JP, et al. Comparison of PAX6 and PAX8 as immunohistochemical markers for pancreatic neuroendocrine tumors. *Endocr Pathol.* 2015 Mar; 26(1):54-62.
3. Walter, RFH. et al. SOX4, SOX11 and PAX6 mRNA expression was identified as a (prognostic) marker for the aggressiveness of neuroendocrine tumors of the lung by using next-generation expression analysis (NanoString). *Future Oncology.* Vol. 11, No. 7, Pages 1027-1036
4. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. *Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories.* Supplement / Vol. 61, January 6, 2012.

## Symbol Key / Légende des symboles/Erläuterung der Symbole

|   |  |   |  |  |   |
|---|--|---|--|--|---|
|  | Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich                        |  | Manufacturer<br>Fabricant<br>Hersteller              |  | Catalog Number<br>Référence du catalogue<br>Bestellnummer |
|  | Read Instructions for Use<br>Consulter les instructions d'utilisation<br>Gebrauchsanweisung beachten |  | Expiration Date<br>Utiliser jusque<br>Verwendbar bis |  | Lot Number<br>Code du lot<br>Chargenbezeichnung           |



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