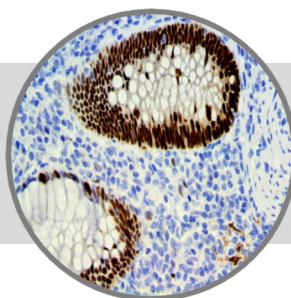


**SOX-9, RMAb****Clone: EP317****Rabbit Monoclonal***Inset: IHC of SOX-9 on a FFPE Colon Tissue*
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**Intended Use**

For In Vitro Diagnostic Use.

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 SOX-9 antibody, clone EP317, 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 SOX9 protein.

**Summary and Explanation**

Transcription factor SOX-9 is a protein that in humans is encoded by the SOX9 gene. SOX9 acts during chondrocyte differentiation and regulates transcription of the anti-Müllerian hormone (AMH) gene. It is expressed during embryogenesis, in the cartilage, neural crest, kidney, and pancreas. SOX-9 plays a pivotal role in male sexual development, interacts with a few other genes to promote the development of male sexual organs and its activity is also required for development, differentiation, and lineage commitment in various tissues including the intestinal epithelium.

SOX9 exhibits several pro-oncogenic properties, including the ability to promote proliferation, inhibit senescence, and collaborate with other oncogenes in neoplastic transformation. Human colorectal cancers show a positive correlation between expression levels of SOX9 and BMI1 and a negative correlation between SOX9 and ARF in clinical samples. In normal colorectal mucosa, SOX9 expression is found predominantly to the lower part of crypts, the proliferative compartment and putative site of stem cells, suggesting SOX9 as a putative stem or progenitor cell biomarker. Recent studies have shown the overexpression of SOX9 in solid tumors. Compared to normal tissues, immunohistochemical analysis revealed staining that is more intense and widespread staining in many cancer types, including but not limited to, Gastric carcinoma, Non-Small Cell Lung Cancer (NSCLC), Lung Adenocarcinoma, Prostate Cancer, Breast Carcinoma, Pancreatic Ductal Adenocarcinoma, Glioma, Colorectal Cancer, Hepatocellular Carcinoma (HCC) and Ovarian Cancer. Amplification of 17q24.3, the chromosomal region of SOX9 has been found in Prostate, Neuroblastoma, Medulloblastoma, Breast and Ovarian Cancer, which all exhibit high SOX9 expression. Although staining is predominantly nuclear, cytoplasmic SOX9 may serve as a valuable prognostic marker for Invasive Ductal Carcinomas and Metastatic Breast Cancer. Additionally, SOX9 upregulation has been associated with higher tumor stage and grade, and overexpression has been recognized as an independent prognostic marker for decreased survival in Colorectal Cancer, NSCLC and HCC patients. In Pancreatic Cancer, SOX9 has been found to regulate the EGFR pathway throughout pancreatic tumorigenesis.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	EP317
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Nuclear	<b>Control</b>	Colon, Prostate, Skin, Breast, Tonsil, Lymph Node, Colon Carcinoma Squamous Cell Carcinoma
<b>Species Reactivity</b>		Human, Predicted: Mouse, Rat	

**Presentation**

SOX-9 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 3211	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 3212	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 3213	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 3214	Concentrated	1:50 - 1:200	0.1 mL
BSB 3215	Concentrated	1:50 - 1:200	0.5 mL
BSB 3216	Concentrated	1:50 - 1:200	1.0 mL

**Control Slides Available**

<i>Catalog No.</i>	<i>Quantity</i>
BSB 3217	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 (NaN<sub>3</sub>) 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. Tommerup N, et al. Assignment of an autosomal sex reversal locus (SRA1) and campomelic dysplasia (CMPD1) to 17q24.3-q25.1. Nat Genet. 1993; 4 (2): 170-4.
2. De Santa Barbara P, et al. Direct interaction of SRY-related protein SOX9 and steroidogenic factor 1 regulates transcription of the human anti-Müllerian hormone gene. Mol. Cell. Biol. 1998; 18 (11): 6653-65.
3. Matheu A, et al. Oncogenicity of the developmental transcription factor Sox9. Cancer Res. 2012; 1;72(5):1301-15.
4. Lü B, et al. Analysis of SOX9 expression in colorectal cancer. Am J Clin Pathol. 2008 Dec; 130 (6):897-904.
5. Chun-Hui Zhou, et al. Clinical significance of SOX9 in human non-small cell lung cancer progression and overall patient survival. J. Exp. Clin.Cancer Res. 2012; 31:18.
6. Grimont A, et al. SOX9 regulates ERBB signalling in pancreatic cancer development. Gut. 2015 Nov; 64(11):1790-9.
7. 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

<b>EC REP</b>	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	<b>REF</b>	Catalog Number Référence du catalogue Bestellnummer
<b>IVD</b>	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum		Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis	<b>LOT</b>	Lot Number Code du lot Chargenbezeichnung



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