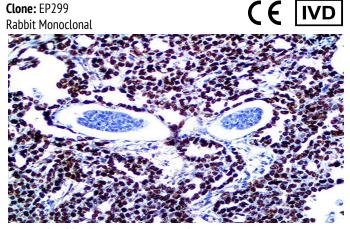


SALL4



Inset: IHC of SALL4 on a FFPE Testicular Cancer Metastasis to Liver Tissue

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.

Immunogen

A synthetic peptide corresponding to residues of human SALL4 protein.

Summary and Explanation

Spalt-like protein 4 (SALL4) is a transcription factor encoded by a member of the Spalt-like (SALL) gene family, SALL4. There are four human SALL proteins (SALL1, 2, 3, and 4) with structural homology and playing diverse roles in embryonic development, kidney function, and cancer. SALL4 expression is low to undetectable in most adult tissues with the exception of germ cells and human blood progenitor cells. In normal testicular tissue, positive, weak SALL4 staining is observed in spermatogonia. In addition, a few (<5%) primary spermatocytes show dot-like weak SALL4 staining. Secondary spermatocytes, spermatids, spermatozoa, and Sertoli cells are negative for anti-SALL4. Leydig cells, rete testis, epididymis, spermatic cord fibroblasts, blood vessels, and hematopoietic cells are negative for SALL4.

SALL4 is reactivated and misregulated in various cancer, such as acute myeloid leukemia (AML), B-cell acute lymphocytic leukemia (B-ALL), germ cell tumors, gastric cancer, breast cancer, hepatocellular carcinoma (HCC). lung cancer, and glioma. In many of these cancers, SALL4 expression has been compared in tumor cells to the normal tissue counterpart, e.g. it is expressed in nearly half of primary human endometrial cancer samples, but not in normal or hyperplastic endometrial tissue samples. Often, SALL4 expression is correlated with worse survival and poor prognosis such as in HCC, or with metastasis such as in endometrial cancer, colorectal carcinoma, and esophageal squamous cell carcinoma. It is unclear how SALL4 expression is deregulated in malignant cells, but DNA hypomethylation in its intron 1 region has been observed in B-ALL. In solid tumors such as germ cell tumors, SALL4 protein expression has become a standard diagnostic biomarker. SALL4 demonstrates 100% sensitivity and stains more than 90% tumor cells in all intratubular germ cell neoplasia, seminomas, dysgerminomas, embryonal carcinomas, and yolk sac tumor (both pediatric and postpubertal). SALL4 is also positive in most cases of teratoma and the mononucleated trophoblastic cells in choriocarcinomas. Most non-testicular tumors from various organs and sites are negative for SALL4, though an

occasional carcinoma or sarcoma may show weak SALL4 staining in less than 25% of tumor cells.

Antibody Type	Rabbit Monoclonal	Clone	EP299		
Isotype	IgG	Reactivity	Paraffin, Frozen		
Localization	Nuclear	Species	Human		
		Reactivity			
Control	Testis, Seminoma, Yolk Sac Tumor				
Application	Ovarian Cancer, Testicular Cancer, Liver Cancer, Breast				
	Cancer, Endometrial and Genital Cancer, Colon and				
	Gastrointestinal Cancer, Germ Cell Tumors,				
	Undifferentiated Tumor				

Presentation

Anti-SALL4 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.

Catalog No.	Presentation	Dilution	Volume	
BSB 3190	Predilute	Ready-to-Use	3.0 mL	
BSB 3191	Predilute	Ready-to-Use	7.0 mL	
BSB 3192	Predilute	Ready-to-Use	15.0 mL	
BSB 3193	Concentrate	1:10-1:50	0.1 mL	
BSB 3194	Concentrate	1:10-1:50	0.5 mL	
BSB 3195	Concentrate	1:10-1:50	1.0 mL	

Control Slides Available

Catalog No.	Quantity		
BSB-9373-CS	5 slides		

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

Precautions

- 1. For professional users only. Results should be interpreted by a qualified medical professional.
- 2. This product contains <0.1% sodium azide (NaN $_3$) as a preservative. Ensure proper handling procedures are used with this reagent.
- 3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
- 4. Dispose of unused solution with copious amounts 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).

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 on acetone-fixed frozen sections and acetone-fixed cell preparations.

IHC Protocol

- 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 IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
- 8. Wash slides with ImmunoDNA washer or DI water.
- 9. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Immunohistochemical Protocol

Step	ImmunoDetecto r 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 Pl0174 or Pl0097.

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. de Celis JF, et al. Regulation and function of Spalt proteins during animal development". The International Journal of Developmental Biology. 2009; 53 (8-10): 1385–98.
- 2. Kohlhase J, et al. SALL4 mutations in Okihiro syndrome (Duane-radial ray syndrome), acro-renal-ocular syndrome, and related disorders. Human Mutation. 2005; 26 (3): 176–83.
- 3. Miettinen M, et al. SALL4 expression in germ cell and non-germ cell tumors: a systematic immunohistochemical study of 3215 cases". The American Journal of Surgical Pathology. 2014; 38 (3): 410–20.
- 4. Zhang X, et al. SALL4: an emerging cancer biomarker and target. Cancer Letters. 2015; 357 (1): 55–62.
- 5. Ueno S, et al. Aberrant expression of SALL4 in acute B cell lymphoblastic leukemia: mechanism, function, and implication for a potential novel therapeutic target. Experimental Hematology. 2014; 42 (4): 307–316.
- 6. Cao D, Li J, et al. SALL4 is a novel diagnostic marker for testicular germ cell tumors. The American Journal of Surgical Pathology. 2009; 33 (7): 1065–77. 7. Zhang L, et al. SALL4, a novel marker for human gastric carcinogenesis and metastasis. Oncogene. 2014; 33 (48): 5491–500.
- 8. Kobayashi D, et al. SALL4 is essential for cancer cell proliferation and is overexpressed at early clinical stages in breast cancer. International Journal of Oncology. 2011; 38 (4): 933–9
- 9. Wei Cui et. al. Differential expression of the novel oncogene, SALL4, in lymphoma, plasma cell myeloma, and acute lymphoblastic leukemia. Modern Pathology, 2006; 19(12), 1585-1592
- 10. 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. https://www.cdc.gov/mmwr/pdf/other/su6101.pdf

Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REF	QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	\	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
IVD	In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro In-Vitro-Diagnostikum	[] i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	\subseteq	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung

