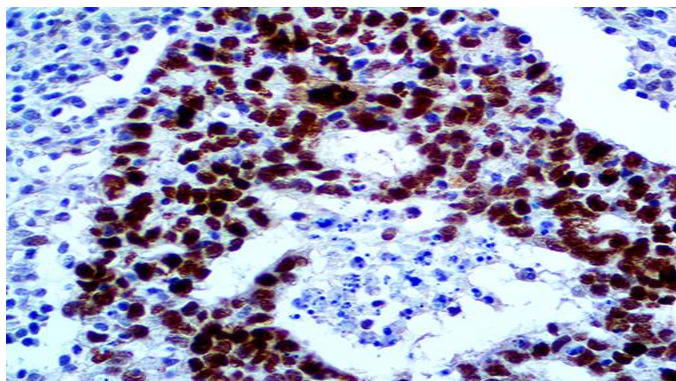


MDM2

Clone: BSB-64
 Mouse Monoclonal



Inset: IHC of MDM2 on a FFPE Liposarcoma 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

Synthetic peptide against the N-terminus of the human MDM2 protein.

Summary and Explanation

MDM2 is a protein that in humans is encoded by the MDM2 gene. MDM2 is an important negative regulator of the p53 tumor suppressor. The MDM2 protein functions both as an E3 ubiquitin ligase that recognizes the N-terminal trans-activation domain (TAD) of the p53 tumor suppressor and an inhibitor of p53 transcriptional activation. The human homologue of this protein is sometimes called HDM2. Further supporting the role of MDM2 as an oncogene, several human tumor types have been shown to have increased levels of MDM2, including soft tissue sarcomas and osteosarcomas as well as breast tumors.

Well Differentiated Liposarcomas (WDLPS), Atypical Lipomatous Tumor/Well-Differentiated Liposarcoma (ALT-WDLPS) and Dedifferentiated Liposarcoma (DDLPS) may be difficult to distinguish from benign Adipose Tumors and from Poorly Differentiated Sarcomas, respectively. Genetically, they are characterized by amplification of MDM2 and CDK4 genes on chromosome 12q13-15. MDM2 and CDK4 protein overexpression have also been identified in these tumors. Detection of MDM2/CDK4 protein overexpression by IHC can be used to diagnose WDLPS and DDLPS. Considering a strong and diffuse immunostaining pattern in most of the neoplastic cells achieves the best results in identifying these tumors. Low-grade Osteosarcoma is a rare malignancy that may be subdivided into two main subgroups on the basis of location in relation to the bone cortex, that is, Parosteal Osteosarcoma and Low-grade Central Osteosarcoma. Their histological appearance is quite similar and characterized by spindle cell stroma with low-to-moderate cellularity and well-differentiated anastomosing bone trabeculae. Immunohistochemical expression of MDM2 and CDK4 is specific and provides sensitive markers for the diagnosis of Low-grade

Osteosarcomas, helping to differentiate them from benign fibrous and fibro-osseous lesions, particularly in cases with atypical radio-clinical presentation and/or limited biopsy samples.

Antibody Type	Mouse Monoclonal	Clone	BSB-64
Isotype	IgG1	Reactivity	Paraffin, Frozen
Localization	Nuclear	Species Reactivity	Human, Mouse, Rat
Control	Testis, Tonsil, Cervix, Placenta, Liposarcoma, Testicular Cancer		
Application	Sarcoma & Soft Tissues, Breast Cancer		

Presentation

Anti-MDM2 is a Mouse 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>Presentation</i>	<i>Dilution</i>	<i>Volume</i>
BSB 2978	Predilute	Ready-to-Use	3.0 mL
BSB 2979	Predilute	Ready-to-Use	7.0 mL
BSB 2980	Predilute	Ready-to-Use	15.0 mL
BSB 2981	Concentrate	1:25-1:100	0.1 mL
BSB 2982	Concentrate	1:25-1:100	0.5 mL
BSB 2983	Concentrate	1:25-1:100	1.0 mL

Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB-9271-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 (NaN₃) 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 Digester (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	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. Oliner JD, et al. Amplification of a gene encoding a p53-associated protein in human sarcomas. *Nature* 1992; 358 (6381): 80-3.
2. Wade M, et al. Hdmx modulates the outcome of p53 activation in human tumor cells. *J. Biol. Chem.* 2006; 281 (44): 33036-44.
3. Aleixo PB, et al. Can MDM2 and CDK4 make the diagnosis of well differentiated /dedifferentiated liposarcoma? An immunohistochemical study on 129 soft tissue tumours. *J Clin Pathol.* 2009; Dec;62(12):1127-35.
4. Binh MB, et al. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data. *Am J Surg Pathol.* 2005; Oct;29(10):1340-7.
5. Fanny Dujardin, et al. MDM2 and CDK4 immunohistochemistry is a valuable tool in the differential diagnosis of low-grade osteosarcomas and other primary fibro-osseous lesions of the bone. *Modern Pathology* 2011; 24, 624-637
6. 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

	QAdvis EAR AB Ideon Science Park Scheelevägen 17 SE-223 70 Lund, Sweden	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
	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	 Lot Number Code du lot Chargenbezeichnung