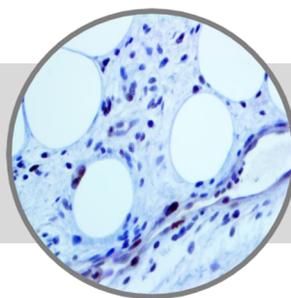


# MDM2

**Clone: BSB-64**

Mouse Monoclonal


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*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 transactivation 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.

<b>Antibody Type</b>	Mouse Monoclonal	<b>Clone</b>	BSB-64
<b>Isotype</b>	IgG1	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Nuclear	<b>Control</b>	Testis, Tonsil, Cervix, Placenta, Liposarcoma, Testicular Cancer
<b>Species Reactivity</b>		Human, Mouse, Rat	

## Presentation

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>Antibody Type</i>	<i>Dilution</i>	<i>Volume/Qty</i>
BSB 2978	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 2979	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 2980	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 2981	Concentrated	1:25 - 1:100	0.1 mL
BSB 2982	Concentrated	1:25 - 1:100	0.5 mL
BSB 2983	Concentrated	1:25 - 1:100	1.0 mL

## Control Slides Available

<i>Catalog No.</i>	<i>Quantity</i>
BSB 2984	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. 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.

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

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands		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

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