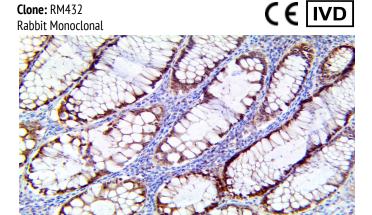


HSP70



Inset: IHC of HSP70 on a FFPE Colon Adenocarcinoma 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

Recombinant human HSP70 protein.

Summary and Explanation

70 kDa heat shock proteins (HSP70) are found ubiquitously in virtually all living organisms, facilitating protein folding and protecting cells from heat stress and toxic chemicals. HSP70 proteins have 3 functional domains: N-terminal ATPase domain, substrate binding domain, and a C-terminal domain that serves as a "lid" for the substrate binding domain. HSP70 binds tightly to partially synthesized peptides and prevents them from aggregating and rendering nonfunctional. HSP70 also inhibits apoptosis by blocking the recruitment of procaspase-9 to the Apaf-1/dATP/cytochrome c apoptosome complex.

HSP70 is shown to be overexpressed in malignant Melanoma and underexpressed in Renal Cell Carcinoma. A variety of tumor cells can express HSP70 with seemingly contradictory functions. Intracellular HSP70 has a cytoprotective function via suppression of apoptosis and lysosomal cell death (LCD) and extracellular HSP70 can promote tumorigenesis and angiogenesis. Other evidence showed intracellular HSP70 can promote apoptosis and membrane-associated/extracellular HSP70 can elicit antitumor innate and adaptive immune responses.

One study evaluated the expression of HSP70, Estrogen Receptor (ER) and Ki-67 and assessed the relationship between them in Cervical Squamous Cell Neoplasia. It found that HSP70 may play an important role in tumor cell proliferation and is more related with invasive Squamous Cell Carcinoma than Cervical Intraepithelial Neoplasia, but ER may be not related with tumor cell proliferation and differentiation. Therefore, HSP70 may be a useful prognostic factor in Cervical Dysplasia and Cancer.

Antibody Type	Rabbit Monoclonal	Clone	RM432		
Isotype	IgG	Reactivity	Paraffin, Frozen		
Localization	Nuclear, Cytoplasmic	Species Reactivity	Human		
Control	Breast, Fallopian Tube, Skin, Prostate, Testis, Transitional Cell Carcinoma				
Application	Cervical Cancer, Melanoma and Skin Cancer, Kidney and Urothelial Cancers				

Presentation

Anti-HSP70 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-3730-3	Predilute	Ready-to-Use	3.0 mL	
BSB-3730-7	Predilute	Ready-to-Use	7.0 mL	
BSB-3730-15	Predilute	Ready-to-Use	15.0 mL	
BSB-3730-01	Concentrate	1:50-1:200	0.1 mL	
BSB-3730-05	Concentrate	1:50-1:200	0.5 mL	
BSB-3730-1	Concentrate	1:50-1:200	1.0 mL	

Control Slides Available

Catalog No.	Quantity		
BSB-3730-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 the 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	ImmunoDetector AP/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. Mayer MP. Gymnastics of molecular chaperones. Mol Cell. 2010;39(3):321-331. doi:10.1016/j.molcel.2010.07.012
- 2. Ricaniadis N, Kataki A, Agnantis N, Androulakis G, Karakousis CP. Long-term prognostic significance of HSP-70, c-myc and HLA-DR expression in patients with malignant melanoma. Eur J Surg Oncol. 2001;27(1):88-93. doi:10.1053/ejso.1999.1018
- 3. Ramp U, Mahotka C, Heikaus S, et al. Expression of heat shock protein 70 in renal cell carcinoma and its relation to tumor progression and prognosis. Histol Histopathol. 2007;22(10):1099-1107. doi:10.14670/HH-22.1099
- 4. Vostakolaei MA, Hatami-Baroogh L, Babaei G, Molavi O, Kordi S, Abdolalizadeh J. Hsp70 in cancer: A double agent in the battle between survival and death [published online ahead of print, 2020 Nov 10]. J Cell Physiol. 2020;10.1002/jcp.30132. doi:10.1002/jcp.30132 5. Kim KK, Jang TJ, Kim JR. HSP70 and ER expression in cervical intraepithelial neoplasia and cervical cancer. J Korean Med Sci. 1998;13(4):383-388. doi:10.3346/jkms.1998.13.4.383
- 6. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe WorkPractices 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	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	\	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

