



Inset: IHC of COX-2 on a FFPE Liver Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalinfixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

* The COX-2 antibody, clone EP293, 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 COX-2 protein.

Summary and Explanation

Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase), also known as cyclooxygenase-2 or COX-2, is an enzyme that in humans is encoded by the PTGS2 gene and it is involved in the conversion of arachidonic acid to prostaglandin H2, an important precursor of prostacyclin and thromboxane A2, among others. Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain; this is the method of action of wellknown drugs such as aspirin and ibuprofen. COX-2 inhibition by nonsteroidal antiinflammatory agents has been shown to decrease angiogenesis and tumor growth, and promote apoptosis.

The expression of COX-2 is upregulated in many cancers. COX-2 overexpression has been associated with increased microvascular density, and VEGF protein expression in head and neck Squamous Cell Carcinomas and is a poor prognostic indicator in this entity as well. The overexpression of COX-2 along with increased angiogenesis and GLUT-1 expression is significantly associated with gallbladder carcinomas. Furthermore the product of COX-2, PGH2 is converted by prostaglandin E2 synthase into PGE2, which in turn can stimulate cancer progression. Consequently inhibiting COX-2 may have benefit in the prevention and treatment of these types of cancer. The mutant allele PTGS2 5939C carriers among the Han Chinese population have been shown to have a higher risk of gastric cancer. In addition, a connection was found between Helicobacter pylori infection and the presence of the 5939C allele. COX-2 overexpression has also been suggested as a poor prognostic indicator in Carcinomas of the Colon, Breast, Pancreas, and Adenocarcinomas of the Lung.

Antibody Type	Rabbit Monoclonal	Clone	EP293	
lsotype	lgG	Reactivity	Paraffin, Frozen	
Localization	Cytoplasmic	Control	Colon, Stomach,	
			Pancreas, Breast,	
			Lung, Colon	
			Adenocarcinoma	
	Species Reactivity	Human, Predicted: Mouse		

Presentation

COX-2 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.	Antibody Type	Dilution	Volume/Qty	
BSB 2873	Tinto Prediluted	Ready-to-Use	3.0 mL	
BSB 2874	Tinto Prediluted	Ready-to-Use	7.0 mL	
BSB 2875	Tinto Prediluted	Ready-to-Use	15.0 mL	
BSB 2876	Concentrated	1:50 - 1:200	0.1 mL	
BSB 2877	Concentrated	1:50 - 1:200	0.5 mL	
BSB 2878	Concentrated	1:50 - 1:200	1.0 mL	

Control Slides Available

Catalog No.	Quantity		
BSB 2879	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 gualified 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 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).

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.

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	

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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. Hla T, Neilson K (August 1992). "Human cyclooxygenase-2 cDNA". Proc. Natl. Acad. Sci. U.S.A. 1992; 89 (16): 7384–8.

2. Legan M., et al. Cyclooxygenase-2, p53 and glucose transporter-1 as predictors of malignancy in the development of gallbladder carcinomas. Bosn J Basic Med Sci . 2010; 10 (3): 192–6.

3. Menter DG, et al. Cyclooxygenase-2 and cancer treatment: understanding the risk should be worth the reward. Clin. Cancer Res. 2010; 16 (5): 1384–90.

4. Li Y, et al. A new cyclo-oxygenase-2 gene variant in the Han Chinese population is associated with an increased risk of gastric carcinoma. Mol Diagn Ther . 2010; 14 (6): 351–5.

5. 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

EC REP	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	2 TO ATC	Storage Temperature Limites de température Zulässiger Temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
	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	\sum	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung



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