



COX-2

IHC of COX-2 on an FFPE Colon Adenocarcinoma Tissue

Description Cyclooxygenase (COX) is an enzyme that is responsible for formation of important biological mediators called prostanoids (including prostaglandins, prostacyclin and thromboxane). Pharmacological inhibition of COX can provide relief from the symptoms of inflammation and pain; this is the method of action of well-known drugs such as aspirin and ibuprofen. COX-2 inhibition by nonsteroidal anti-inflammatory agents has been shown to decrease angiogenesis and tumor growth, and promote apoptosis.

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. 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	SP21
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Adenocarcinoma of Colon
Storage	Store at 2°-8°C	Stability	2 years

For long-term storage of the concentrated antibody, it is recommended that aliquots of the antibody be frozen at -20°C in glycerol 50% (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Dilute using an antibody diluent such as ImmunoDetector Protein Block/Antibody Diluent (BSB 0040 and BSB 0041) or ImmunoDNA Background Blocker (BSB 0103-BSB 0107).

Presentation Anti-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.

Availability	Catalog No.	Antibody Type	Dilution	Volume/QTY
	BSB 5358	Prediluted	Ready-To-Use	3.0 ml
	BSB 5359	Prediluted	Ready-To-Use	7.0 ml
	BSB 5360	Prediluted	Ready-To-Use	15.0 ml
	BSB 5361	Concentrated	1:25-1:100	0.1 ml
	BSB 5362	Concentrated	1:25-1:100	0.5 ml
	BSB 5363	Concentrated	1:25-1:100	1.0 ml
	BSB 5364	Control Slides		5

Note: For concentrated antibodies, please centrifuge prior to use to ensure recovery of all product.

- References**
1. Stoehlmacker J, Lenz Heinz-Josef, *Semin Oncol* 30.(3)suppl6(June),2003:10-16
 2. Gallo O, et al. *Hum Pathol.* 33:708-714
 3. Sano H, et al. *Cancer Res.* 1995;Sep 1;55(17):3785-9
 4. Denkert C, et al. *Cancer.* 2003;Jun 15;97(12):2978-87
 5. Sheehan KM, et al. *Hum Pathol.* 34:1242-1246

Protocol Suggested protocol on reverse

Recommended Immunohistochemical Protocol

- Pretreatment**
1. Cut and mount 3-4 micron formalin-fixed paraffin-embedded tissues on positive charged slides.
 2. Air dry for 2 hours at 58° C.
 3. Deparaffinize, dehydrate and rehydrate tissues.
 4. Subject tissues to heat epitope retrieval 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. **Electric Pressure Cooker**
Place standoff rack at base of pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high, and incubate for 15 minutes. Open and immediately transfer slides to room temperature.
 - b. **Water Bath Method**
Place tissues/slides in a pre-warmed staining dish or coplin jar containing the **ImmunoDNA Retriever with Citrate** or **EDTA** in a water bath set 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. Wash slides with IHC wash buffer or DI water.
 8. Continue IHC staining protocol.

Immunohistochemical Protocol

Step	ImmunoDetector (AP or HRP)	PolyDetector (AP or HRP)
Peroxidase/AP Block	5 minutes	5 minutes
Primary Antibody	30 minutes	45 minutes
Secondary Biotinylated Link	10 minutes	Not Applicable
AP or HRP Label	10 minutes	45 minutes
Substrate-Chromogen	5-10 minutes	10 minutes
Counterstaining	Time varies with counterstain	Time varies with counterstain

