



ALK-1

IHC of ALK-1 on an FFPE Anaplastic Large Cell Lymphoma Tissue

Description Anaplastic Lymphoma Kinase (ALK) was originally discovered as a NPM (Nucleophosmin)-ALK fusion protein. The ALK gene is on chromosome 2. Upon translocation between chromosome 2 and chromosome 5 t(2;5), the ALK gene fuses with the NPM gene. The chimeric product (NPM ALK) resulting from t(2;5) translocation is a protein of 80 kDa with the N terminal portion of NPM linked to the complete intracellular portion of ALK.

This antibody recognizes a human p80 protein, identified as a hybrid of the Anaplastic Lymphoma Kinase (ALK) gene and the Nucleophosmin (NPM) gene resulting from the t(2;5)(p23;q35) translocation found in a third of Large-Cell Lymphomas. ALK-1 is detected in 60% of Anaplastic Large-Cell Lymphomas and has proven to indicate a better prognosis in the ALK-1 (+) group.

Antibody Type	Rabbit Monoclonal	Clone	SP8
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic, Nuclear	Control	Anaplastic Large Cell Lymphoma
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-ALK-1 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 5043	Prediluted	Ready-To-Use	3.0 ml
	BSB 5044	Prediluted	Ready-To-Use	7.0 ml
	BSB 5045	Prediluted	Ready-To-Use	15.0 ml
	BSB 5046	Concentrated	1:50-1:200	0.1 ml
	BSB 5047	Concentrated	1:50-1:200	0.5 ml
	BSB 5048	Concentrated	1:50-1:200	1.0 ml
	BSB 5049	Control Slides		5

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

- References**
1. Cataldo KA, et al. *Am J Surg Pathol*. 1999;32(1):1386-1392.
 2. Nakamura S, Shiota M, et al. *Am J Surg Pathol*. 1997;21(12):1420-1432.
 3. Falini B, Bigerna B, et al. *Am J Pathol*. 1998; 153(3)Sept. 875-886.

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

