



## Bax

### *IHC of Bax on an FFPE Hodgkin's Lymphoma Tissue*

**Description** Bax is a protein of the bcl-2 gene family. It promotes apoptosis by competing with bcl-2 proper. The Bax gene contains a small promoter element that complements a binding domain on the multi-faceted p53 tumor suppressor. Wild-type p53 has been demonstrated to upregulate the transcription of a chimeric reporter plasmid, utilizing the consensus promoter sequence of Bax approx. 50-fold over mutant p53. Mutations in this consensus sequence eliminate transcription of the reporter gene. Thus, it is likely that p53 promotes Bax's apoptotic faculties in vivo as a primary transcription factor.

Bax exerts a pro-apoptotic rather than an anti-apoptotic effect on cells. Bax targets mitochondrial membranes, inducing mitochondrial damage and cell death in a caspase-independent manner. Bad plays a critical role in the Bax-mediated apoptosis pathway by dimerizing with BclxL, causing the displacement of Bax. The displacement of Bax allows apoptosis to proceed.

<b>Antibody Type</b>	Mouse Monoclonal	<b>Clone</b>	SPM336
<b>Isotype</b>	IgG1/K	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Cytoplasmic and Cell Membrane	<b>Control</b>	Hodgkin's Lymphoma, Normal Breast, Tonsil
<b>Storage</b>	Store at 2°-8°C	<b>Stability</b>	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** Bax 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.

Availability	Catalog No.	Antibody Type	Dilution	Volume/QTY
	BSB 6078	Prediluted	Ready-To-Use	3.0 ml
	BSB 6079	Prediluted	Ready-To-Use	7.0 ml
	BSB 6080	Prediluted	Ready-To-Use	15.0 ml
	BSB 6081	Concentrated	1:50-1:200	0.1 ml
	BSB 6082	Concentrated	1:50-1:200	0.5 ml
	BSB 6083	Concentrated	1:50-1:200	1.0 ml
	BSB 6084	Control Slides		5

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

**References**

- Oltvai Z, et al. *Cell*. 1993;74,609-619
- Miyashita T, Reed J, *Cell*. 1995;80,293-299

**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

