



## CD4

*IHC of CD4 on an FFPE Tonsil Tissue*

**Description** CD4 is a glycoprotein expressed on the surface of T-helper cells, regulatory T-cells, monocytes, macrophages, and dendritic cells. On T-cells, CD4 is the co-receptor for the T-cell receptor (TCR). It amplifies the signal generated by the TCR by recruiting the tyrosine kinase that is essential for activating many molecules involved in the signaling cascade of an activated T-cell.

CD4 antigen is involved in the recognition of Type II Major Histocompatibility Complex antigens (MHC-II). CD4 is also the receptor for Human Immunodeficiency Virus (HIV). It is present on most T-helper cells and normal thymocytes.

<b>Antibody Type</b>	Rabbit Monoclonal	<b>Clone</b>	SP35
<b>Isotype</b>	IgG	<b>Reactivity</b>	Paraffin, Frozen
<b>Localization</b>	Membranous	<b>Control</b>	Tonsil, Lymph Node
<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** CD4 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 5148	Prediluted	Ready-To-Use	3.0 ml
	BSB 5149	Prediluted	Ready-To-Use	7.0 ml
	BSB 5150	Prediluted	Ready-To-Use	15.0 ml
	BSB 5151	Concentrated	1:50-1:200	0.1 ml
	BSB 5152	Concentrated	1:50-1:200	0.5 ml
	BSB 5153	Concentrated	1:50-1:200	1.0 ml
	BSB 5154	Control Slides		5

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

- References**
- Stein H, et al. *Adv Cancer Res.* 1984;42:67-147.
  - Abbas AK, Lichtman AH, *Cellular and Molecular Immunology (5th Ed.)* 2003

**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

