



Cyclin D3

IHC of Cyclin D3 on an FFPE Breast Carcinoma Tissue

Description Cyclin D3, also known as CCND3, is a human gene. The protein encoded by this gene belongs to the highly-conserved cyclin family, whose members are characterized by a dramatic periodicity in protein abundance through the cell cycle. Cyclins function as regulators of Cdk kinases. This cyclin forms a complex with and functions as a regulatory subunit of Cdk4 or Cdk6, whose activity is required for cell-cycle G1/S transition. This protein has been shown to interact with and be involved in the phosphorylation of tumor suppressor protein Rb. The Cdk4 activity associated with this cyclin was reported to be necessary for cell-cycle progression through G2 phase into mitosis after UV radiation.

In normal adult tissues, Cyclin D3 shows two patterns of distribution: in lymphoid tissues it is expressed in proliferative compartments, while in most other tissues it is expressed by terminally differentiated/quiescent cells. In non-Hodgkin's Lymphomas, Cyclin D3 immunolabelling correlates with proliferative activity and progression. Benign endocrine tumors are frequently strongly Cyclin D3-positive, while high-grade (Small-cell) Neuroendocrine Carcinomas are always negative. In Breast Carcinomas, no relationship has been seen between ER status and Ki67 labelling. In soft tissue neoplasms, Cyclin D3 is consistently expressed in some tumors, such as Stromal Tumors of the Gastrointestinal Tract and Embryonal Rhabdomyosarcomas.

Antibody Type	Mouse Monoclonal	Clone	SPM241
Isotype	IgG1/K	Reactivity	Paraffin, Frozen
Localization	Nuclear	Control	Tonsil, Breast Carcinomas
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 Cyclin D3 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 6106	Prediluted	Ready-To-Use	3.0 ml
	BSB 6107	Prediluted	Ready-To-Use	7.0 ml
	BSB 6108	Prediluted	Ready-To-Use	15.0 ml
	BSB 6109	Concentrated	1:50-1:200	0.1 ml
	BSB 6110	Concentrated	1:50-1:200	0.5 ml
	BSB 6111	Concentrated	1:50-1:200	1.0 ml
	BSB 6112	Control Slides		5

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

References

1. Doglioni C, et al. *J Pathol.* 1998;185(2):159-66
2. Dowdy SF, et al. *Cell.* 1993;73(3):499-511
3. Lin J, et al. *Oncogene.* 2001;20(16):2000-2009

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

