Specimen Sheet

Please note
Some information is Lot dependent.

MONOCLONAL MOUSE
ANTI-Cyclin B1
CODE NO.: M3530
LOT NO.: 098

Immunogen: Purified hamster cyclin B1
Clone: V152
Presentation: Anti-hamster Cyclin B1 is a mouse monoclonal antibody supplied in liquid form as purified tissue culture supernatant, in 0.05 M Tris-HCl, pH 7.2, and 15 mM sodium azide.

Protein Concentration: 1.1 mg/mL (Refractometry)
Mouse IgG Concentration: 196 µg/mL (Single Radial Immunodiffusion)
Subclass: IgG1, kappa

Precautions: 1. For In Vitro Diagnostic Use.

2. Contains sodium azide as a preservative, which is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing.

Specificity: Cyclin B1 is a member of the cyclin family of proteins which are selectively expressed at different phases of the cell cycle. Their synthesis and degradation are tightly regulated and loss of regulation is thought to play a role in malignant transformation. Cyclins associate with and activate cyclin-dependent kinases (CDKs), targeting them to key substrates. CDK-mediated phosphorylation of distinct groups of proteins propels the cell through the different phases and checkpoints of the cell cycle. Cyclin B1 is a G2 cyclin which complexes with protein kinase p34cdc2 to regulate the onset of mitosis. Synthesis of cyclin B1 is initiated during early G2, where the protein resides in the cytoplasm. The protein is translocated to the nucleus during prophase, its maximal expression occurring in late G2/M, and is degraded during anaphase. Anti-hamster Cyclin B1, clone V152 (anti-Cyclin B1) has been shown to react with mouse and human cyclin B1 in immunohistochemistry and also identifies the protein in immunoblots.

Reactivity: Normal cells: Actively proliferating cells in normal tissue express cyclin B1. Cyclin B1 has been localized by immunohistochemistry (IHC) in formalin-fixed, paraffin-embedded breast tissue to the cytoplasm and to some nuclei of subsets of luminal and proliferative epithelial cells in normal breast glands. Cyclin B1 expression has been demonstrated by flow cytometry in phytohemagglutinin stimulated normal peripheral blood lymphocytes which were determined to be in very late S and in the G2+M phases of the cell cycle. In a study of breast lesions, the mean proliferative

(over)
index for cyclin B1 (PI_{cyclin B1}), was determined by computerized static image analysis and compared with that of normal mammary glands. The mean PI_{cyclin B1} of breast carcinomas was demonstrated to be 4.3-fold higher than that of normal glands.\(^4\)

In an IHC study of formalin-fixed, paraffin-embedded lymphomas, cyclin B1 was shown to be expressed by high grade lymphomas and positivity correlated with MIB1 expression. The highest level of cyclin B1 expression was found in Burkitt’s lymphoma. Also positive were diffuse large cell lymphomas and, more weakly, high grade MALT lymphomas.\(^5\) Cyclin B1 has been demonstrated by Western blot analysis in the majority of metastatic melanomas, but not in benign nevi.\(^7\) Colorectal cancers were also evaluated by Western blotting and reported to express higher levels of cyclin B1 than non-neoplastic mucosa.\(^6\)

Staining Procedure

**Paraffin sections**

Anti-Cyclin B1 can be used on formalin-fixed, paraffin-embedded tissue sections. The deparaffinized tissue sections must be treated with heat prior to the IHC staining procedure. For greater adherence of tissue sections to glass slides, the use of silanized slides (DAKO® Code No. S3003) is recommended.

When using the water bath method, preheat a Coplin jar containing 10 mM citrate buffer, pH 6.0 as well as a water bath to 95-99°C. When the temperature has stabilized, place tissue sections into the coplin jar containing the preheated buffer. Heat the tissue sections for 40 minutes. For improved staining results and a shorter incubation time, DAKO® Target Retrieval Solution (Code No. S1700) can be used in place of the 10 mM citrate buffer. Under these conditions the incubation time in the water bath may be reduced to 20 minutes. After thermal treatment, allow the jar with buffer and slides to cool for 20 minutes at room temperature. Rinse well with distilled water and place slides into buffer (Tris, PBS, etc.).

Anti-Cyclin B1 may be used at a dilution of 1:200-1:400 in the LSAB+ method determined on formalin-fixed, paraffin-embedded tissue. For diluting the antibody, DAKO® Antibody Diluent with Background Reducing Components (Code No. S3022) is recommended. These are guidelines only; optimal dilutions should be determined by the individual laboratory.

**Cryostat Sections and Cell Smears**

Anti-Cyclin B1 can also be used to label cryostat sections or cell smears.

Storage

Store at 2-8°C or -20°C. Avoid repeated freeze-thaw cycles.

References