

Methods for Detection of Cyclin D1 Protein using MAb to Cyclin D1 (CD1.1) (Prod. No. ALX-804-539):

IMMUNOHISTOCHEMISTRY:

Tissue processing:

Tissue should be fixed as soon as possible after removal. The choice of fixative is critical (the staining does not perform from methacarn). From formalin fixation it is essential to use antigen retrieval (boiling in pressure cooker, micro-waving or water bath in 0.001 M EDTA-NaOH buffer, pH 8.0). In general, the antigen retrieval condition must be set up individually for each laboratory and equipment (we currently apply 30 minutes at 97°C at heated magnetic stirrer). The silanized or electrostatically charged slides (e.g. Menzel Superfrost Plus) are necessary to prevent section detachment.

Incubation and concentration of antibody:

The working dilution is 0.1-1 µg/ml in PBS or TBS (pH 7.4-7.6), containing 0.5-1% of immunohistochemistry grade BSA. The DAKO antibody diluent is suggested for this purpose, too. Incubation for 2 hours at 25-37°C or overnight incubation at 4-8°C is recommended. We suggest setting up the antibody dilution individually again (currently we use tissue culture supernatant diluted 1:3'000 with overnight incubation and DAKO DAB⁺ detection).

Detection:

A sensitive streptavidin (avidin)/biotin-peroxidase system is recommended for detection. Commercially available detection kits perform well, we suggest Vector Elite ABC with DAKO DAB⁺.

Additional steps:

5% non-fat dry milk in PBS for 30 minutes is recommended for non-specific binding activity blocking. 3% hydrogen peroxide in PBS for 10 minutes will optionally block the peroxidase activity. We recommend following the instructions supplied together with the detection kit.

Staining results and controls:

A nuclear staining pattern plus a faint diffuse cytoplasmic staining. Breast carcinomas, colon polyps and mantle zone lymphomas tend to overexpress this protein. The proper combination of antigen retrieval time and temperature, antibody concentration, incubation time and detection sensitivity must be set up carefully and individually to visualize differences in the expression level between cell types and/or during cell cycle.

GEL ELECTROPHORESIS AND IMMUNOBLOTTING:

Solubilized proteins were separated by SDS-PAGE on 12.5% gel and transferred onto a nitrocellulose membrane in a Bio-Rad Mini Trans-Blot Electrophoretic Transfer Cell for 2 hours, at 4°C and 140 mA in transfer buffer (25 mM TRIS, 190 mM glycine and 20% methanol).

The blots were blocked in 5% milk-PBST (PBST = PBS + 0.1% Tween 20) for 1 hour, probed either overnight at 4°C or for 2 hours at room temperature with monoclonal antibody and washed 3x in PBST.

The blots were then incubated for 1 hour at room temperature in horseradish peroxidase (HRP)-conjugated rabbit anti-mouse immunoglobulin antiserum (DAKO) diluted 1:1'000 (in 5% milk-PBST), and washed as above.

The peroxidase activity was detected by enhanced chemiluminescence (ECL) reaction using Hyperfilm - MP (Amersham).