

PN IM1740 Cyclin D1 (5D4)

Liquid, 0.1 mL
0.1 mg

For Research Use Only. Not For Use In Diagnostic Procedures

SPECIFICITY

Cyclin D1, also called Bcl-1, CCND1, or PRAD1, is a 36 kDa protein which regulates cyclin-dependent protein kinase activity in the G1 phase of the cell cycle (1, 2). The antibody recognizes human cyclin D1, D2 and mouse cyclin D1, D2. It does not recognize human cyclin D3, neither mouse cyclin D3.

REAGENT

Unconjugated monoclonal antibody
Cyclin D1 (Bcl-1), liquid, 0.1 mL, PN IM1740

Clone	5D4
Hybridoma	PAI x Balb/c
Immunogen	Human PRAD1/Cyclin D1 gene product from E. coli.
Ig Chain	IgG2a
Species	Mouse
Source	Ascites fluid
Purification	Affinity chromatography on protein A.

BUFFER

This antibody is provided in phosphate-buffered saline (PBS), containing 50% glycerol.

APPLICATION

Immunoblotting.
Immunohistochemistry.
Staining patterns, as well as staining intensity, of the cyclin D1 antibody may vary, depending on the type and state of the tissue evaluated. Both nuclear and cytoplasmic staining have been observed.

STATEMENT OF WARNINGS

- This reagent contains 50% glycerol. Glycerol is irritating for eyes and skin. Wear suitable protective clothing. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
- Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
- Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
- Do not use reagent beyond the expiration date on the vial label.
- Avoid microbial contamination of reconstituted reagent or erroneous results may occur.
- Use Good Laboratory Practices (GLP) when handling this reagent.

STORAGE CONDITIONS AND STABILITY

This antibody is provided in liquid form and must be stored at -20°C until the expiration date stated on the vial label.
No preservative has been added.

REAGENT PREPARATION

Aliquoting is suggested to avoid multiple freeze-thaw cycles. To prepare the working solution (1 to 10 $\mu\text{g}/\text{mL}$), dilute the monoclonal antibody in PBS containing 1% BSA.

SUGGESTED WORKING CONCENTRATION

Immunoblotting: 1 to 10 $\mu\text{g}/\text{mL}$
Immunohistochemistry: 1 to 10 $\mu\text{g}/\text{mL}$

PROCEDURE FOR IMMUNOHISTOCHEMISTRY

NOTE: this reagent may be used for frozen or paraffin sections. Paraffin sections should be pre-processed using heat treatment in a microwave oven, a pressure cooker or other heating equipment*.

Heat or enzymatic treatment may induce loss of adherence of tissue sections to glass slides. This may be overcome by using glass slides coated according to either procedure a) or b).

a) Gelatin-Chrome Alum Dip Method (3):

Dilute 2.5 g of gelatin and 2 g of $\text{KCr}(\text{SO}_4)_2 \cdot 12\text{H}_2\text{O}$ in 500 mL of distilled water at $40 - 50^{\circ}\text{C}$.

Slides should be dipped into this solution for 1 second and allowed to dry at room temperature.

b) Aminoalkylsilane (APES) (4):

Dilute 1 mL of 3-aminopropyltriethoxysilane in 50 mL of acetone.

Slides should be dipped into this solution for 20 seconds, then washed twice in acetone and twice in distilled water. Tissue sections are floated onto slides in a water bath, prior to being dried overnight at 37°C or at room temperature for 48 hours.

Procedure with microwave oven treatment

Prepare a 10 mM citrate buffer, pH 6.0 (5) using:

- stock solution A: 0.1M citric acid (store at $2 - 8^{\circ}\text{C}$),
- stock solution B: 0.1M sodium citrate (store at $2 - 8^{\circ}\text{C}$),

by mixing 9 mL of A with 41 mL of B and add distilled water to a final volume of 500 mL.

This 10 mM citrate buffer is available through Beckman Coulter® using PN IM1975.

Deparaffinized slides should be placed in a thermo-resistant dish filled with the 10 mM citrate buffer. Run 3 - 5 cycles of 5 minutes each in a 750 watt microwave oven. Boiling is normally observed. Refill the dish with distilled water to replace evaporated water. Sections should not dry out. To avoid bubbles trapped between slides, it is recommended to leave at least 4 mm between slides.

Remove the dish from the oven and allow to cool down for 20 minutes at room temperature. Rinse slides in TBS buffer.

Immunostain according to previously described methods (6): the antibody should be incubated on tissue sections for 60 minutes at room temperature.

Procedure with pressure cooker treatment

Prepare a 10 mM citrate buffer, pH 6.0 (5) using:

- stock solution A: 0.1M citric acid (store at $2 - 8^{\circ}\text{C}$),
- stock solution B: 0.1M sodium citrate (store at $2 - 8^{\circ}\text{C}$),

by mixing 36 mL of A with 164 mL of B and add distilled water to a final volume of 2000 mL.

This 10 mM citrate buffer is available through Beckman Coulter® using PN IM1975.

Bring 2000 mL of 10 mM citrate buffer to a boil in a stainless steel pressure cooker, using an electric hot plate. Cover, but do not lock lid during that step. When buffer is boiling, immerse deparaffinized slides totally in buffer. Lock the lid. Let the air vent/cover and overpressure plug rise for about 4 minutes. Remove pressure cooker from heat source and run under cold water with lid on. When the small valve drops, open lid. DO NOT OPEN LID UNTIL THE Vent Cover Lock Drops or Pressure Indicator shows that the pressure has been released (see Instructions for your own particular pressure cooker). Remove slides and wash sections with a slow flow of tap water for about 1 minute. Then wash sections in TBS buffer for 2 x 5 minutes.

Immunostain according to previously described methods (6): the antibody should be incubated on tissue sections for 60 minutes at room temperature.

SELECTED RESEARCH REFERENCES

- Xiong, Y., Connolly, T., Fletcher, B., Beach, D., "Human D-type cyclin", 1991, Cell, 4, 65, 691-699.
- Motokura, T., Bloom, T., Kim, H.G., Juppner, H., Ruderman, J.V., Kronenberg, H.M., Arnold, A., "A novel cyclin encoded by a bc11-linked candidate oncogene", 1991, Nature, 6318, 350, 512-515.
- Luna, L.G., "Staining controls, section adhesives and tissue artifacts", 1992, Histopathologic methods and color atlas for special stains and tissue artifacts, American Histolabs, Gaithers, American Histolabs, Inc., Publications Division, 563-593.
- Henderson, C., "Aminoalkylsilane: an inexpensive, simple preparation for slide

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- adhesion", 1989, J. Histotechnol., 12, 123-124.
5. Szekeres, G., "Detection of the Ki-67 antigen in fixed proliferating cells", 1993, Anal. Cell. Pathol., 5, 249-250.
 6. Leong, A.S.Y., "Immunohistochemistry: theoretical and practical aspects", 1993, Applied Immunohistochemistry for the Surgical Pathologist, Edward Arnold, London, 2-22.

* Depending on the exact protocol followed, this step may require a license under U.S. Patent 5,244,787

Manufactured for:
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