

# Cyclin D1

Concentrated and Prediluted Rabbit Monoclonal Antibody  
901-432-030222

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CME 432 A, C	0.1, 1.0 mL	1:100	Renoir Red
Predilute	PME 432 AA	6.0 mL	Ready-to-use	N/A
ONCORE	OAI 432 T60	60 tests	Ready-to-use	N/A
ONCORE Pro	OPAI 432 T60	60 tests	Ready-to-use	N/A
Q Series— For Leica BOND-III	ALI 432 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

Cyclin D1 [EP12] is a rabbit monoclonal antibody that is intended for laboratory use in the qualitative identification of cyclin D1 protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

## Summary and Explanation:

This rabbit monoclonal antibody recognizes a protein of 36 kDa, identified as Cyclin D1 (also known as Bcl-1 or PRAD-1). Cyclin D1 is a regulatory subunit of certain protein kinases thought to advance the G1 phase of the cell cycle (5). Cyclin D1 used in tandem with CD5, CD10 and CD23 is the most reliable immunohistochemical marker for mantle cell lymphoma. Cyclin D1 is also expressed in invasive breast cancer (6). Due to the superior technology in the development of this antibody, its binding capacity is superior to mouse monoclonal antibodies and is virtually background free (1).

## Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Rabbit monoclonal

**Species Reactivity:** Human; others not tested

**Clone:** EP12

**Isotype:** IgG

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** Cyclin D1

**Cellular Localization:** Nuclear

**Positive Tissue Control:** Mantle cell lymphoma and breast cancer

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Supplied As:** Buffer with protein carrier and preservative

## Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Borg or Reveal Decloaker. Refer to the Borg or Reveal Decloaker product data sheet for specific instructions.

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Probe:** N/A

## Protocol Recommendations (intelliPATH FLX and manual use)

### Cont'd:

**Polymer:** Incubate for 30 minutes at RT with a secondary-conjugated polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB - OR - Incubate for 5-7 minutes at RT with Warp Red.

### Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

### Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## Protocol Recommendations (ONCORE™ Automated Slide Staining System):

OAI432 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** Cyclin D1 Rb

**Protocol Template (Description):** Rb HRP Template 1

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

**Reagent Name, Time, Temp.:** Cyclin D1 Rb, 30 min., 25°C

## Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI432 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** Cyclin D1 Rb

**Protocol Template (Description):** Rb HRP Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** Cyclin D1 Rb, 30 min., 25°C

## Protocol Recommendations (Q Series – For Leica BOND-III):

ALI432 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

**Protocol Name:** IHC Protocol F

**Detection:** Bond Polymer Refine

**HIER:** 40 min with ER2

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary:** 8 min

**Polymer:** 8 min

**Mixed DAB Refine:** 10 min

**Hematoxylin:** 5 min

## Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the

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## Limitations Cont'd:

superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

## Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA ([www.clsi.org](http://www.clsi.org)). 2011

## Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN<sub>3</sub>) used as a preservative 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. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (9)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. (10)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

## Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

## References:

1. Pruneri G, *et al.* SP4, a novel anti-cyclin D1 rabbit monoclonal antibody, is a highly sensitive probe for identifying mantle cell lymphomas bearing the t(11;14)(q13;q32) translocation. *Appl Immunohistochem Mol Morphol.* 2005 Dec; 13(4):318-22.
2. de Leon ED, *et al.* Usefulness of an immunohistochemical panel in paraffin-embedded tissues for the differentiation of B-cell non-Hodgkin's lymphomas of small lymphocytes. *Mod Pathol.* 1998 Nov; 11(11):1046-51.
3. Samaha H, *et al.* Mantle cell lymphoma: a retrospective study of 121 cases. *Leukemia.* 1998 Aug; 12(8):1281-7.
4. Quintanilla-Martinez L, *et al.* Mantle cell lymphomas lack expression of p27Kip1, a cyclin-dependent kinase inhibitor. *Am J Pathol.* 1998 Jul; 153(1):175-82.
5. Nakamura S, Yatabe Y, Seto M. Cyclin D1 overexpression in malignant lymphomas. *Pathol Int.* 1997 Jul; 47(7):421-9.
6. van Diest PJ, *et al.* Cyclin D1 expression in invasive breast cancer. Correlations and prognostic value. *Am J Pathol.* 1997 Feb; 150(2):705-11.
7. de Boer CJ, *et al.* Cyclin D1 protein analysis in the diagnosis of mantle cell lymphoma. *Blood.* 1995 Oct 1; 86(7):2715-23.
8. Bartkova J, *et al.* Cell cycle-related variation and tissue-restricted expression of human cyclin D1 protein. *J Pathol.* 1994 Mar; 172(3):237-45.
9. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
10. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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