OriGene Technologies, Inc.
9620 Medical Center Drive, Ste 200
Rockville, MD 20850, US
Phone: +1-888-267-4436
https://www.origene.com
techsupport@origene.com
EU: info-de@origene.com
CN: techsupport@origene.cn

Product datasheet for AM26674AF-N

Cyclin D1 (CCND1) Mouse Monoclonal Antibody [Clone ID: 5D4]

Product data:

Product Type:	Primary Antibodies
Clone Name:	5D4
Applications:	FC, IHC, IP, WB
Recommended Dilution:	Western blot: 1 µg/mL for chemiluminescence detection system. Immunoprecipitation: 1-10 µg/200 µg of cell extract from $5x10^6$ cells. Immunohistochemstry on frozen sections: 1-10 µg/mL; Heat treatment is necessary for paraffin embedded sections. Microwave oven; 2 times for 10 minutes each in 10 mM citrate buffer (pH 6.5). Flow cytometry: 10 µg/mL (final concentration). For details see protocol below.
Reactivity:	Human, Mouse
Host:	Mouse
Isotype:	IgG2a
Clonality:	Monoclonal
Immunogen:	Recombinant human PRAD1/Cyclin D1
Specificity:	This antibody reacts with human Cyclin D1 (36 kDa). This clone 5D4 recognizes human Cyclin D1, D2 and mouse Cyclin D1, D2, but not human and mouse Cyclin D3.
Formulation:	PBS containing 50% glycerol, pH 7.2 State: Azide Free State: Liquid Ig fraction without preservatives
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	cyclin D1
Database Link:	Entrez Gene 595 Human P24385

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Background: Cyclin D1, also known as Bcl-1, CCND1, or PRAD-1, is a 36 kDa nuclear protein that plays a key regulatory role during the G1 phase of the cell cycle by regulating the activity of Cdk proteins. Cyclin D1 forms a complex with Cdk4, then binds to and phosphorylates Rb protein, triggering cells to progress from G0/G1 to S and thus driving cellular proliferation. Cyclin D1 protein overexpression is found in the majority of human breast cancers and is usually linked with poor prognosis. Overexpression of cyclin D1 is a well-established criterion for the diagnosis of Mantle Cell Lymphoma, and also has been used to distinguish malignant breast carcinomas from premalignant breast lesions.

Synonyms: Cyclin-D1, PRAD-1 oncogene, BCL-1 oncogene, CCND1, BCL1, PRAD1

Note: This product was originally produced by MBL International.

Protocol:
SDS-PAGE & Western Blottin
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl, pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
6) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the APPLICATIONS for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with the 1:10,000 HRP-conjugated anti-mouse IgG (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
13) Expose to an X-ray film in a dark room for 3 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.
(Positive controls for Western blotting; ZR-75-1, MCF7, HeLa, WR19L, NIH/3T3)

Immunoprecipitation
1) Wash the cells 3 times with PBS and suspend with 10 volume of cold Lysis buffer (50 mM Tris-HCl pH 7.2, 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol) containing appropriate protease inhibitors. Incubate it at 4oC with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC and transfer the supernatant to another tube.
3) Add primary antibody as suggest in the APPLICATIONS into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4oC.
4) Add 20 µL of 50% protein A agarose resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4oC.
5) Wash the beads 3-5 times with the cold Lysis buffer (centrifuge the tube at 2,500 x g for 10 seconds).
6) Resuspend the beads in 20 µL of Laemmli's sample buffer, boil for 3-5 minutes, and centrifuge for 5 minutes.
7) Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
8) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm2 for 1 hour in a semi-dry transfer system (Transfer Buffer: 25 mM Tris, 190 mM glycine, 20% MeOH). See the manufacture's manual for precise transfer procedure.
9) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4oC.
10) Incubate the membrane with 0.1 µg/mL of polyclonal antibody Anti-Cyclin D1 (MBL; code no. 553) as primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
11) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
12) Incubate the membrane with the 1:10,000 HRP-conjugated anti-rabbit IgG (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
13) Wash the membrane with PBS-T (5 minutes x 6 times).
14) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
15) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.
(Positive control for Immunoprecipitation; ZR-75-1)

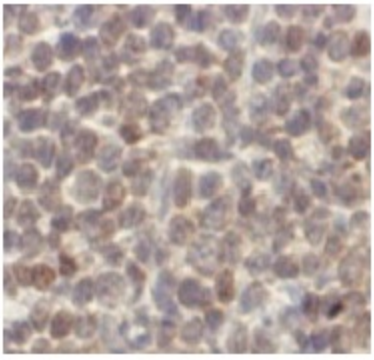
Immunohistochemical staining for paraffin-embedded sections: SAB method
1) Deparaffinize the sections with Xylene 3 times for 3-5 minutes each.
2) Wash the slides with Ethanol 3 times for 3-5 minutes each.
3) Wash the slides with PBS 3 times for 3-5 minutes each.
4) Heat treatment Heat treatment by Microwave: Place the slides put on staining basket in 500 mL beaker with 500 mL of 10 mM citrate buffer (pH 6.5). Cover the beaker with plastic wrap, then process the slides 2 times for 10 minutes each at 500 W with microwave oven. Let the slides cool down in the beaker at room temperature for about 40 minutes.

5) Remove the slides from the citrate buffer and cover each section with 3% H2O2 for 10 minutes at room temperature to block endogenous peroxidase activity. Wash 3 times in PBS for 5 minutes each.
6) Remove the slides from PBS, wipe gently around each section and cover tissues with Protein Blocking Agent for 5 minutes to block non-specific staining. Do not wash.
7) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS containing 1% BSA as suggest in the APPLICATIONS.
8) Incubate the sections for 1 hour at room temperature.
9) Wash the slides 3 times in PBS for 5 minutes each.
10) Wipe gently around each section and cover tissues with Polyvalent Biotinylated Antibody (Ultratech HRP Kit). Incubate for 15 minutes at room temperature. Wash as in step 9).
11) Wipe gently around each section and cover tissues with Streptavidin-Peroxidase. Incubate for 15 minutes at room temperature. Wash as in step 9).
12) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H2O2 in 150 mL PBS. *DAB is a suspect carcinogen and must be handled with care. Always wear gloves.
13) Wash the slides in water for 5 minutes.
14) Counter stain in hematoxylin for 1 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
15) Now ready for mounting.
(Positive controls for Immunohistochemistry; ZR-75-1, mantle cell lymphoma)

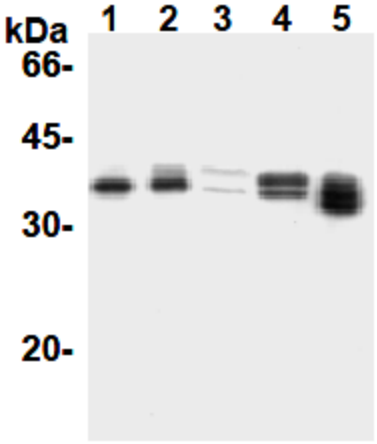
Flow cytometric analysis for cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Add 200 µL of 4% paraformaldehyde (PFA)/PBS to the cell pellet after tapping. Mix well, then fix the cells for 15 minutes at 4oC.
3) Wash the cells 3 times with washing buffer.
4) Add 200 µL of 70% ethanol to the cell pellet after tapping. Mix well, then permeablize the cells for 30 minutes at -20oC.
5) Wash the cells 3 times with washing buffer.
6) Add 20 µL of Clear Back (human Fc receptor blocking reagent) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
7) Add 40 µL of the primary antibody at the concentration of as suggest in the APPLICATIONS diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 30 µL of 1:100 FITC conjugated anti-mouse IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

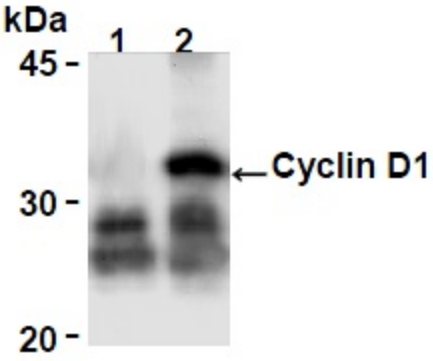
Product images:



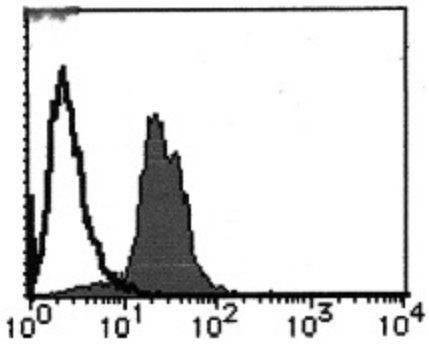
Immunohistochemical detection of Cyclin D1 on paraffin embeddedsection of Human Mantle Cell Lymphoma with AM26674AF-N.



Western blot analysis of Cyclin D1 expression in ZR-75-1 (1), MCF7 (2), HeLa (3), WR19L (4) and NIH/3T3 (5) using AM26674AF-N.



Immunoprecipitation of Cyclin D1 from ZR-75-1 cells with Mouse IgG2a (1) or AM26674AF-N (2). After immunoprecipitated with the antibody, immunocomplex was resolved on SDSPAGE and immunoblotted with 553.



Flow cytometric analysis of Cyclin D1 expression in HeLa cells. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of AM26674AF-N to the cells.