Novocastra™ Lyophilized Mouse Monoclonal Antibody Cyclin D1

Product Code: NCL-CYCLIN D1

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human cyclin D1 protein.
Clone	DCS-6
Ig Class	IgG2a
Antigen Used for Immunizations	Prokaryotic recombinant fusion protein corresponding to the human cyclin D1 molecule.
Hybridoma Partner	Mouse myeloma (NS-2).
Preparation	Lyophilized tissue culture supernatant containing 15 mM sodium azide. Reconstitute with the volume of sterile distilled water indicated on the vial label.
Effective on Frozen Tissue	Yes
Effective on Paraffin Wax Embedded Tissue	Yes (using 1 mM EDTA (pH 8.0) or 1 mM EDTA/10 mM Tris (pH 9.0) unmasking solution combined with the high temperature antigen unmasking technique: see overleaf, or trypsin digestion of sections).
Recommendations on Use	Immunohistochemistry: Typical working dilution 1:20. High temperature antigen unmasking technique or trypsin digestion of sections is required. The choice of antigen unmasking technique should be determined by the individual laboratory. 60 minutes primary antibody incubation at 25 °C. Standard ABC technique. Western Blotting: Typical working dilution 1:100–1:250.
Positive Controls	Immunohistochemistry: Formalin-fixed, paraffin-embedded WI-38 cell line (diploid human fibroblast cell line). Western Blotting: ZR75 cell line.
Staining Pattern	Nuclear, however cytoplasmic staining may also be seen, especially in formalin-fixed tissues.
Storage and Stability	Store unopened lyophilized antibody at 4 °C. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label. The reconstituted antibody is stable for at least two months when stored at 4 °C. For long term storage, it is recommended that aliquots of the antibody are frozen at -20 °C (frost-free freezers are not recommended). Repeated freezing and thawing must be avoided. Prepare working dilutions on the day of use.
General Overview	The D-type cyclins are a family of proteins which function primarily by regulating the activity of cyclin dependent protein kinases in the G1 phase of the cell cycle. Cyclin D1, also known as (PRAD-1 or bcl-1) is a 36 kD protein. Maximum expression of cyclin D1 occurs at a critical point in mid to late G1 phase of the cell cycle.
General References	Camacho F I, Garcia J F, Sánchez-Verde L, et al.. American Journal of Pathology. 158 (4): 1363–1369 (2001). Holley S L, Parkes G, Matthias C, et al.. American Journal of Pathology. 159 (5): 1917–1924 (2001). Takes R P, Baatenburg de Jong R J, Wijffels K, et al.. Journal of Pathology. 194: 298–302 (2001). Hayashi H, Ito T, Yazawa T, et al.. Journal of Pathology. 192: 26–31 (2000). Kohmura T, Hasegawa Y, Ogawa T, et al.. Archive Otolaryngology Head Neck Surgery. 125 (12): 1351–1354 (1999). Betticher D C, Heighway J, Thatcher N, et al.. British Journal of Cancer. 75 (12): 1761–1768 (1997). Bartkova J, Lukas J, Müller H, et al.. International Journal of Cancer. 57: 353–361 (1994). Bartkova J, Lukas J, Strauss M, et al.. Journal of Pathology. 172: 237–245 (1994). Gillett C, Fantl V, Smith R, et al.. Cancer Research. 54: 1812–1817 (1994). Lukas J, Bartek J and Strauss M. Journal of Immunological Methods. 170: 255–259 (1994). Lukas J, Müller H, Bartkova J, et al.. Journal of Cell Biology. 125: 625–638 (1994). Lukas J, Pagano M, Staskova Z, et al.. Oncogene. 9: 707–718 (1994). Müller H, Lukas J, Schneider A, et al.. Proceedings of the National Academy of Sciences USA. 91: 2945–2949 (1994).

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Instructions for Use

High Temperature Antigen Unmasking Technique Followed by Trypsin Digestion for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf - see other Epitope Retrieval Solutions in the range) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Place slides in pre-heated distilled water to bring the sections to 37 °C for a minimum of 5 minutes.
9. Incubate sections in pre-heated Trypsin solution at 37 °C for 30 seconds.
10. Rinse sections in running tap water.
11. Proceed with immunohistochemistry protocol.
12. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
13. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
14. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate sections in an appropriate biotinylated secondary antibody.
17. Wash in TBS buffer for 2 x 5 minutes.
18. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
19. Wash in TBS buffer for 2 x 5 minutes.
20. Incubate slides in DAB or other suitable peroxidase substrate.
21. Wash thoroughly in running tap water.
22. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

Trypsin Solution

**Trypsin containing chymotrypsin should always be used. The enzyme activities can vary from a supplier and between batches. Such variations may affect the incubation time required.*

Preheat the following to 37 °C using a water bath:

(i) 200 mL of TBS

(ii) 200 mL of distilled water.

Dissolve 0.2 g Trypsin 250 and 0.2 g Calcium Chloride in the 200 mL of TBS.

Once the Trypsin solution is at 37 °C, pH to 7.8 with 1 M sodium hydroxide.

0.01 M Citrate Buffer (pH 6.0) or RE7113 (where available).

Add 3.84 grams of Citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available)

Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 L of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/0.65 mM EDTA/0.0005% TWEEN (pH 9.0) or RE7119 (where available)

Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ THE MANUFACTURER'S INSTRUCTIONS.